42nd Congress of the International Society of Paediatric Oncology
Boston, USA, October 21 - 24, 2010

SIOP EDUCATION BOOK 2010

International Society of Paediatric Oncology

www.siop.nl

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Bharat R. Agarwal
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SOCIÉTÉ INTERNATIONALE D'ONCOLOGIE PÉDIATRIQUE
SIOP
INTERNATIONAL SOCIETY OF PAEDIATRIC ONCOLOGY

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SIOP

INTERNATIONAL SOCIETY
OF PAEDIATRIC ONCOLOGY

Vision

No child should die of cancer.

Mission

The mission of the International Society of Paediatric Oncology (SIOP) is:

- To ensure that each child and young adult with cancer has access to state of the art treatment and care.
- To ensure that all involved in childhood cancer worldwide, have access to the latest progress through meetings, networking, and continuing professional development.
- To support those caring for children and young adults with cancer to provide the best curative and palliative therapies.
- To advocate for appropriate long term follow up for children and young adults after treatment for cancer.

Goals

- To define the essential facilities and resources for delivery of child cancer care.
- To help care givers develop and identify resources necessary to build adequate infrastructure for local delivery of cancer care.
- In recognition of the disparity of resource and access, to assist in adaptation of treatment to improve outcome regardless of location.
- To work with all relevant agencies to ensure uniform availability of effective and affordable anticancer and supportive care agents.
- To organize regional and global meetings for those involved in childhood cancer to provide a platform for education, research, and professional networking.
- To have over 50% of countries represented in the SIOP membership.
- To have over 50% of pediatric oncologists worldwide as members of SIOP.
- To have 50% of active members attend the annual meeting.
- To ensure that annual meetings are affordable to all members.
- To facilitate continuing professional education beyond that provided in SIOP's meetings.
- To secure the Society's financial stability.

SIOP Council
February 2008
Adopted by SIOP General Assembly, October 2008

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First published 2010

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Acknowledgements

The International Society of Paediatric Oncology would like to acknowledge the efforts of the authors. SIOP is immensely grateful to all the authors for not only presenting state of the art lectures and occasional sessions at the meeting but for agreeing to produce a manuscript to assist in ongoing education for participants at the 42nd meeting of SIOP in Boston 2010.

Disclaimer

The contents of this book represent the views of the individual authors and not necessarily of SIOP.

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Preface : SIOP EDUCATION BOOK 2010

Dear members, dear friends of SIOP,

We are delighted to provide you with this years' Educational Book. At this 42nd meeting of the International Society of Paediatric Oncology here in Boston the Educational Session is focussing on the largest group op patients we treat: Acute Lymphoblastic Leukemia. By reading the different chapters, we realize lots of different aspects are covered by the top players in the field. This year Keynote Lectures show a whole spectrum of key research development within intervention, but also in epidemiology and psycho-oncology. Futures and successes in treatment with either drugs or in the field of stem cell transplantation are provided. Like always, we are grateful to the authors and presenters that they took the time to provide us and you with these overviews. These papers will water your mouth and will make you eager for their presentations!

We hope you will enjoy this year's book as much as the previous ones.

With kind personal regards,

Maarten Egeler
President, SIOP

Lisa Diller
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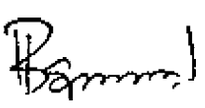

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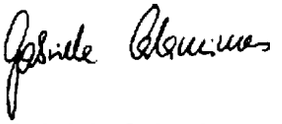


Gabriele Calaminus
Chair, Scientific Committee,
President Elect, SIOP

P r e f a c e : SIOP EDUCATION BOOK 2009

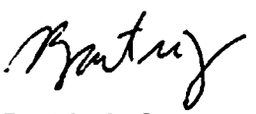
Dear members, dear friends of SIOP,

Like the last couple of years, also at the 41st meeting of the International Society of Pediatric Oncology here in Sao Paolo, we are happy to welcome you here and to provide you with this years' Educational Book. The Educational session is focussing on every aspect possible of Soft Tissue Sarcomas, from epidemiology, diagnosis and the different treatment option to molecular pathogenesis and gene expression. This year Keynote Lectures show certain specifics of the many malignant diseases we see daily in our practise. Again we are extremely grateful to the presenters that they took the time to provide us and you with these manuscripts which can be seen as a contribution to your educational and professional development, which the Council of SIOP considers as one of the main purposes of our society.

We hope you will enjoy this year's book as much as the previous ones.

With kind personal regards,

Maarten Egeler
President, SIOP



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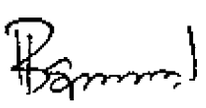

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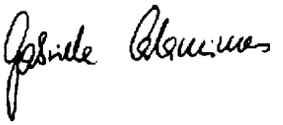

Gabriele Calaminus
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SIOP

Preface : SIOP EDUCATION BOOK 2008

The people behind the International Society of Paediatric Oncology welcome you all here in Berlin for the 40th Annual Meeting. Together with the Scientific Committee and the Local Organizing Committee, we have created an exciting program, of which the keynote and State-of-the Art lectures are published in this SIOP Education Book 2008. We are grateful to all the presenters for their time and effort to provide us and you with these manuscripts, which can be seen as a contribution to your educational and professional development.

We have been told over and over by many of you about the value of the earlier Education Books. This feedback to our Secretariat helps us in understanding your desires and needs. We hope you will enjoy this year's book as much as the previous ones.

Maarten Egeler
President, SIOP

Gunter Henze
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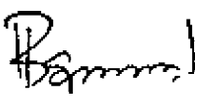

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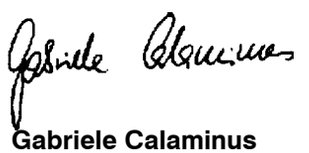

Gabriele Calaminus
Chair, Scientific Committee,
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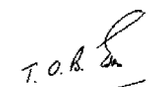
Preface : SIOP EDUCATION BOOK 2007

Welcome to the 39th Annual SIOP Congress here in Mumbai. For the third year running the keynote and State of the Art lecturers have very kindly provided papers to supplement their talks to provide delegates with a reference text for continuing profession education and development. The response from the authors has been tremendous and we are most grateful once again to them for this extra contribution to the meeting. We hope that you will all find this a very useful supplement to the meeting. Feedback on its value would be appreciated. Meanwhile on behalf of the local organisers, scientific committee and board can we wish you a very enjoyable, educating and inspiring conference?



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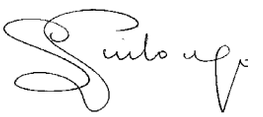

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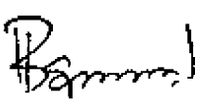

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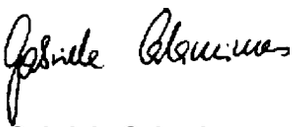

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Preface : SIOP EDUCATION BOOK 2006

At each SIOP meeting we attempt to bring together many of those who are working in the field of paediatric haematology and oncology worldwide to share our experiences and our expertise. SIOP has gradually developed in recent years an increasing educational component to the meeting including specific pre-meeting educational sessions and a series of keynote lectures and state of the art talks. In 2005 we put those talks together in an educational book which we have tried to make available to those who obviously attend the meeting but also worldwide to members and those who have access to the website. I am most grateful to those who agreed to talk and present their papers that they are willing to contribute to this important educational document. We hope that those who can attend the lectures and those who can't but are able to read this book find it useful and of course educational. The book demonstrates the wide breadth of content of current SIOP meetings. It is a good advertisement for the annual meeting. If you are reading this book and are not a member you can see why you should become one.

Enjoy the book and the talks..

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38th Congress of SIOP

Preface : SIOP EDUCATION BOOK 2005

On behalf of the local organizers of the 37th Congress of the International Society of Paediatric Oncology, the Board and Scientific Committee of SIOP we would like to thank the authors for their presentations and for inclusion to this educational book along with the participants who contributed searching questions and informed comments to all of the educational sessions. This is a new venture for SIOP and is warmly welcomed by the members. Professional education is one of the key components of the SIOP meeting. We are delighted that we have had the opportunity in the wonderful surroundings of Vancouver to be able to provide an increasing component of education to the meeting. SIOP and the Education Committee have put a lot of effort into trying to create the right environment for exchange of information and knowledge. We hope that whoever reads this text will benefit from it. We planned this as an experiment this year and we hope that it will become a permanent fixture of SIOP meetings. We of course would appreciate feed back on the value of the text any comments on how we can improve the educational component of the meeting for future years. Good reading and best wishes.

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SECTION A

INTERNATIONAL SOCIETY
OF PAEDIATRIC ONCOLOGY

Childhood Acute Lymphoblastic Leukemia: Currently Applied Prognostic Factors

Lewis B. Silverman

Abstract

Several clinical and biologic factors have been found to be significant predictors of outcome in childhood acute lymphoblastic leukemia (ALL), including age, presenting leukocyte count, immunophenotype, recurrent chromosomal abnormalities, and response to initial therapy as assessed by light microscopy as well as more sensitive measures of submicroscopic disease. Over the last several decades, these prognostic factors have been used to stratify therapy; patients with "high risk" features have received more intensive treatments, while those with features associated with a lower risk of relapse receive less aggressive therapy. This article will review those factors that are currently utilized by clinical trials groups world-wide to determine treatment for children and adolescents with ALL.

Introduction

Over the last sixty years, there has been a dramatic improvement in the outcome of children with acute lymphoblastic leukemia (ALL). With current treatment regimens, event-free survival rates now approach or exceed 80%.(1-3) This success was achieved, in part, through the implementation of risk-stratified therapy. Patients presenting with features that are associated with a higher risk of relapse receive more intensive treatment, while those with features linked to a more favorable outcome are treated with more modest, less toxic therapy.

Risk-based treatment assignment requires the identification of prognostic factors that reliably predict outcome. For children with ALL, a number of clinical and laboratory features have demonstrated prognostic significance, including age, presenting leukocyte count, immunophenotype, lymphoblast chromosomal abnormalities (such as ploidy and translocations), CNS status at diagnosis, and the rapidity with which patients respond to initial induction chemotherapy.(4) The goal of risk-stratified therapy is to "treat away" the adverse prognostic significance of various leukemia subtypes, so that even "high-risk" patients achieve favorable rates of cure. Ultimately, the prognostic significance of any factor is treatment-dependent, and the relevance of any particular factor must be evaluated within the context of the administered therapeutic regimen.

The prognostic factors that are currently applied by most clinical trials groups in the design and implementation of risk-stratified protocols for children with newly diagnosed ALL are summarized in Table 1. These include:

1. Age

The age of patients with ALL significantly correlates with clinical outcome. In childhood ALL, infants and adolescents have a worse prognosis than patients aged 1-10 years.(5) The superior outcomes of children aged 1-10 years is at least partly explained by the high frequency of more favorable underlying biologic features in the lymphoblasts of patients in this age group, including high hyperdiploidy (51-65 chromosomes) and the *TEL/AML1* (also known as *ETV6-RUNX1*) fusion.(5, 6)

ALL in infancy (< 12 months at diagnosis) is associated with high presenting leukocyte counts, increased frequency of central nervous system leukemia at presentation and a very high incidence (~80%) of rearrangements of the *MLL* gene on chromosome 11q23.(7, 8) Infants whose lymphoblasts lack *MLL* gene rearrangements have a significantly better prognosis than those with this chromosomal abnormality.(7, 9) Amongst infants with *MLL* gene rearrangements, those presenting at a young age (< 6 months) or with extremely high leukocyte counts (≥ 300, 000/mL) appear to

have the worst prognosis.(7) *MLL*-rearranged infants are usually treated with more intensive therapies than children aged 1-10 years, often including agents not typically administered to older children with ALL, such as high-dose cytarabine.(7)

Adolescents (ages 10-21 years) with ALL also have a less favorable outcome than children aged 1-10 years, although not as poor as infants. Compared with younger children, adolescents with ALL more frequently present with T-cell immunophenotype, high presenting leukocyte counts, a lower incidence of favorable cytogenetic abnormalities (eg, high hyperdiploidy and the *TEL/AML1* gene fusion) and a higher incidence of the Philadelphia chromosome [t(9;22)].(5, 10) Adolescents also appear to be at higher risk for certain treatment-related complications, such as osteonecrosis, pancreatitis and deep vein thromboses, (10, 11) which may also impact prognosis. On most pediatric protocols, adolescents are considered high risk, regardless of other presenting features. A number of retrospective studies published over the last decade suggest that adolescents

Table 1: Prognostic Factors Currently Used to Determine Therapy by a sample of Childhood ALL Clinical Trials Group

	BFM	COG	DCOG	DFCI	FRALLE	St. Jude	TPOG
Age	No*	Yes	No*	Yes	Yes	Yes	Yes
WBC	No	Yes	No	Yes	Yes	Yes	Yes
Phenotype	Yes	Yes	No	Yes	Yes	Yes	Yes
Sex	No	Yes**	No	No	No	No	Yes**
CNS Status	Yes	Yes	No	Yes	Yes	Yes	Yes
Early Morph. Response	Yes	Yes	Yes	No	Yes	No	Yes
MRD	Yes	Yes	Yes	Yes	Yes	Yes	Yes
TEL/AML1	Yes	Yes	No	No	No	Yes	Yes
Hyperdiploidy; favorable trisomies	No	Yes	No	No	No	Yes	Yes
Hypodiploidy	Yes	Yes	No	Yes	Yes	Yes	Yes
Ph+	Yes	Yes	Yes	Yes	Yes	Yes	Yes
MLL rearrangement	Yes^{+}	Yes	Yes^{+}	Yes	Yes	Yes	Yes
t(1;19)	No	No	No	No	No	Yes	Yes
iAMP21	No	No	No	No	Yes	No	No
Testicular involvement	No	Yes	No	No	No	Yes	Yes

* Except infants; **Duration of therapy only; $^{+}$t(4;11) only

Group Name:

- **BFM**: Berlin-Frankfurt-Munster Study Group/Italian Association of Pediatric Hematology and Oncology
- **COG**: Children's Oncology Group
- **DCOG**: Dutch Childhood Oncology Group
- **DFCI**: Dana-Farber Cancer Institute ALL Consortium
- **FRALLE**: French Acute Lymphoblastic Leukemia Group
- **St. Jude**: St. Jude Children's Research Hospital
- **TPOG**: Taiwan Pediatric Oncology Group

[Personal communication from Martin Schrappe (BFM/AIOEP), Stephen Hunger (COG), Rob Pieters (DCOG), Andre Baruchel (FRALLE), Ching-Hon Pui (St. Jude), Der-Cherng Liang (TPOG)]

achieve better outcome if treated using high-risk pediatric regimens rather than adult ALL protocols.(12, 13)

2. Leukocyte Count

Along with age, the initial peripheral blood leukocyte count was one of the first identified prognostic factors in childhood ALL. Presenting leukocyte count has continued to be an independent predictor of outcome in recent studies, even when controlling for minimal residual disease levels and/or prognostically significant chromosomal abnormalities, (14, 15) and so is still a component of risk group determination on most regimens. Since 1996, based upon guidelines developed by the Cancer Therapy Evaluation Program (CTEP) of the National Cancer Institute (NCI), a leukocyte count of 50, 000/mL has typically been used as the cut-off to classify patients as either high risk or low/standard risk.(4)

3. Immunophenotype

Approximately 80-85% of childhood ALL has a B-lymphoblastic (B-precursor) phenotype, while 10-15% has a T-cell immunophenotype. Historically, patients with T-ALL had an inferior outcome, but when treated more intensively, these children appear to fare as well as those with B-precursor phenotype.(16) Thus, patients with T-ALL are excluded from the low-risk arm of many ALL regimens, and are treated more intensively either on high-risk arms or on separate protocols.

In approximately 15-30% of patients with newly diagnosed ALL, flow cytometry reveals co-expression of at least one myeloid antigen on the cell surface of the lymphoblasts. Myeloid antigen co-expression has been associated with several chromosomal abnormalities, both favorable and unfavorable, including the *TEL/AML1* fusion [t(12;21)], *MLL* gene rearrangements, and the Philadelphia chromosome [t(9;22)], but is almost never observed in high hyperdiploid ALL (>50 chromosomes).(17) Myeloid antigen co-expression is no longer considered an independent predictor of outcome in childhood ALL, (17, 18) and it is not currently a factor in the determination of risk group status on most regimens.

4. CNS Status at Diagnosis

Approximately 15-20% of children with ALL present with detectable lymphoblasts in their cerebrospinal fluid (CSF).(19) Some patient subsets, such as infants and those with T-cell ALL, have a higher incidence of CNS leukemia at diagnosis.(5) CNS status at diagnosis has been correlated with outcome, and is used by most clinical trials groups to determine the intensity of both systemic and CNS-directed therapies.

CNS status at presentation is standardly classified as CNS-1 (no blast cells in the CSF), CNS-2 (fewer than five leukocytes per microliter with blast cells), and CNS-3 (more than five leukocytes per microliter with blast cells or cranial nerve palsy). Patients with CNS-3 status at diagnosis appear to have a higher risk of both CNS and marrow relapses, and so are typically treated with more intensive CNS-directed therapies and often more intensive systemic chemotherapy as well.(19, 20)

CNS-2 status at diagnosis has also been associated with an inferior outcome (although not as unfavorable as CNS-3 status). However, the adverse prognostic significance of CNS-2 status appears to be overcome by the administration of more doses of intrathecal therapy, especially early in therapy, without intensification of systemic therapy.(19, 20) Thus, CNS-2 status is, in general, used as a determinant of CNS-directed therapy but usually does not change a patient's risk group status. A traumatic lumbar punctures with lymphoblasts at diagnosis has also been associated with an increased risk of subsequent CNS relapse; (19, 21) patients with this feature are often treated with intensified CNS-directed treatments, including additional doses of intrathecal chemotherapy, and sometimes with intensified systemic therapy as well.

5. Chromosomal Abnormalities.

Many recurrent chromosomal abnormalities have been reported to have prognostic significance in childhood ALL, and several are utilized in current regimens to risk-stratify patients. Most of the cytogenetic abnormalities used to stratify treatment are much more common in B-precursor ALL than in T-ALL. Thus,

it is predominantly patients with B-precursor ALL who have therapy changed because of a chromosomal abnormality. While multiple chromosomal aberrations with possible prognostic significance have been identified in T-ALL, they are not currently considered by most groups when stratifying therapy.

Two chromosomal abnormalities, high hyperdiploidy (51-65 chromosomes or DNA index greater than or equal to 1.16) and the *TEL/AML1* fusion [t(12;21)], have been associated with a more favorable prognosis. (14, 15) Both of these abnormalities are more common in non-adolescent/non-infant children (i.e., those aged 1-10 years) with B-precursor phenotype.(5, 6) The most favorable outcomes in high hyperdiploid ALL have been associated with the presence of trisomies of chromosomes 4, 10 and 17.(22, 23) Up to 80% of children with B-precursor ALL diagnosed between the ages 2-7 years have either high hyperdiploidy or the *TEL/AML1* fusion, (6) although never both; these two chromosomal abnormalities appear to be mutually exclusive.(24) Some groups will alter therapy based upon the presence of either high hyperdiploidy (and/or favorable trisomies) or *TEL/AML1*; patients with one of these abnormalities (often in conjunction with other favorable presenting features, such as age between 1-10 years, low leukocyte count and rapid response to initial therapy as determined by morphology or minimal residual disease levels) are given less intensive, potentially less toxic treatment.(23)

Chromosomal abnormalities associated with an adverse prognosis currently utilized by most groups to classify patients as “higher risk” include hypodiploidy (fewer than 44-45 chromosomes), (25) rearrangements of the *MLL* gene on chromosome 11q23 (especially t(4;11) translocation), (26) and the Philadelphia chromosome [t(9;22)].(27, 28) Patients with the Philadelphia chromosome are often treated on separate clinical trials which include intensive myelosuppressive chemotherapy, tyrosine kinase inhibitors and/or allogeneic stem cell transplantation in first remission.(28, 33)

6. Early Morphologic Response to Initial Chemotherapy

The rapidity with which a patient responds to initial chemotherapy is a significant predictor of long-term outcome. Treatment stratification for protocols of the Berlin-Frankfurt-Muenster (BFM) and other groups is, in part, based on the absolute peripheral blood blast count measured after a steroid prophase consisting of one dose of intrathecal methotrexate and 7-days of prednisone given immediately prior to the initiation of multiagent induction chemotherapy.(2) Patients with an absolute blast count less than 1, 000/μL at the end of the prophase (a good prednisone response) have a more favorable prognosis than do patients whose peripheral blast counts remain above 1, 000/μL (a poor prednisone response).(2) On studies conducted by the BFM and AIEOP groups, prednisone prophase response is an independent predictor of outcome, even when controlling for other prognostic factors such as minimal residual disease levels and chromosomal abnormalities.(14)

Morphological persistence of marrow disease 7 or 14 days following initiation of multiagent induction chemotherapy also correlates with long-term outcome. Patients with fewer than 5% marrow lymphoblasts as detected by light microscopy at these early time points have a more favorable prognosis than those who disease persists at higher levels.(29) Based on trials which demonstrated that intensification of therapy could abrogate the adverse prognostic significance of slow early morphologic marrow response, (30) patients with this feature receive more intensified post-induction treatment on some clinical trials.

Persistence of microscopically visible leukemia at the end of the first month of induction chemotherapy, observed in up to 5% of children with ALL, is associated with a very poor outcome.(31, 32) While the majority of the patients with initial induction failure will ultimately achieve complete remission, the risk of subsequent relapse is very high and such patients are typically treated with more intensive therapies, including allogeneic stem cell transplant in first remission.(33)

7. Minimal Residual Disease (MRD)

MRD evaluation is a more sensitive measure of early treatment response than assessments based on light microscopy. Submicroscopic

levels of disease can be measured using the polymerase chain reaction (targeting lymphoblast-specific immunoglobulin or T-cell receptor gene rearrangements, or chromosomal translocations) or specialized multiparemeter flow cytometry. Using these techniques, leukemia cells have been identified at levels as low as 1/1000 to 1/100, 000 cells. (14, 34, 35)

Multiple studies have demonstrated that end-induction MRD is an important, independent predictor of outcome in children with ALL.(14, 35, 36) Patients with higher levels of end-induction MRD have a poorer prognosis than those with lower or undetectable levels. MRD at end-induction is used by almost all groups as a factor to determine the intensity of post-induction treatment, with patients found to have higher levels allocated to more intensive therapies regardless of other presenting features. MRD levels at earlier (e.g., Days 8 and 15 of induction) and later time points (e.g., Day 78 of therapy) also predict long-term outcome.(14, 36, 37) For example, the BFM group uses both end-induction (day 33) and Day 78 measurements to risk-stratify patients.(14)

In addition to identifying patients who might benefit from more intensive therapy, MRD assessments, in conjunction with other presenting features, may also be used to identify patient subsets with extremely low risk of relapse. For instance, the Children's Oncology Group (COG) reported that standard-risk B-precursor patients with favorable cytogenetic abnormalities (such as the TEL-AML1 fusion and trisomies of chromosomes 4 and 10) as well as MRD negativity at both Day 8 and at the end of remission induction had a particularly favorable prognosis.(36)

8. Other Prognostic Factors

The following are other prognostic factors which are not universally applied for risk stratification, but are used by individual clinical trial groups when determining intensity or duration of therapy:

- *Sex*: In some studies, the prognosis for boys with ALL is slightly worse than it is for girls.(29) This difference in outcome cannot be entirely explained by the frequency of testicular relapses, which, on current regimens, is quite low. Because of this outcome difference, on some regimens, such as those of the Children's Oncology Group, boys receive a longer maintenance phase than girls, resulting in a longer total duration of treatment. However, on many other regimens, boys and girls receive the same duration of treatment.
- *Overt Testicular Involvement at diagnosis*: Overt testicular involvement at the time of diagnosis occurs in approximately 2% of males. Historically, testicular involvement at diagnosis was identified as an adverse prognostic factor, but its prognostic relevance is less clear on more recent studies.(38, 39) Overt testicular involvement at diagnosis is still considered a high-risk feature in some ALL treatment programs.
- *t(1;19) translocation (TCF3-PBX1 or E2A-PBX1)*: The t(1;19) translocation occurs in approximately 5% of childhood ALL cases, and has previously been associated with inferior outcome.(40) More recently, the results of several clinical trials have suggested that the t(1;19) translocation is not an independent predictor of outcome.(15) The presence of this translocation is considered a high-risk feature in some trials, such as those currently being conducted by the St. Jude Children's Research Hospital, (20) but it is not a determinant of risk group status for most other clinical trials groups.
- *Intrachromosomal amplification of chromosome 21 (iAMP21)*: This abnormality, characterized by multiple extra copies of the *AML1 (RUNX1)* gene on a single chromosome 21, occurs in fewer than 5% of precursor-B cell ALL.(41) It has been associated with an inferior outcome, (41) and is considered a high-risk feature on some clinical trials.

Summary and Future Directions

Several clinical and biologic features have been found to have important prognostic significance in childhood ALL, including age, presenting leukocyte count, immunophenotype, CNS status, recurrent chromosomal abnormalities, and response to initial therapy. The application of risk-stratified therapy utilizing these prognostic

factors has resulted in long-term event-free survival in up to 80-85% of children with ALL. Further improvement in outcome will require, in part, the discovery of novel prognostic factors to identify the 15-20% of patients who are not cured with current therapies. Recent advances in our understanding of underlying leukemia biology, including the identification of prognostically distinctive subsets of patients, and of host pharmacogenomics may allow for more precise risk stratification and more targeted, individualized treatment planning.

References

1. Silverman LB, Stevenson KE, O'Brien JE, Asselin BL, Barr RD, Clavell L, et al. Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985-2000). Leukemia. 2010 Feb;24(2):320-34.
2. Moricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, Gadner H, et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia. 2010 Feb;24(2):265-84.
3. Pui CH, Pei D, Sandlund JT, Ribeiro RC, Rubnitz JE, Raimondi SC, et al. Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. Leukemia. 2010 Feb;24(2):371-82.
4. Smith M, Arthur D, Camitta B, Carroll AJ, Crist W, Gaynon P, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol. 1996;14(1):18-24.
5. Moricke A, Zimmermann M, Reiter A, Gadner H, Odenwald E, Harbott J, et al. Prognostic impact of age in children and adolescents with acute lymphoblastic leukemia: data from the trials ALL-BFM 86, 90, and 95. Klin Padiatr. 2005 Nov-Dec;217(6):310-20.
6. Forestier E, Schmiegelow K. The incidence peaks of the childhood acute leukemias reflect specific cytogenetic aberrations. J Pediatr Hematol Oncol. 2006 Aug;28(8):486-95.
7. Pieters R, Schrappe M, De Lorenzo P, Hann I, De Rossi G, Felice M, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. Lancet. 2007 Jul 21;370(9583):240-50.
8. Hilden JM, Dinndorf PA, Meerbaum SO, Sather H, Villaluna D, Heerema NA, et al. Analysis of prognostic factors of acute lymphoblastic leukemia in infants: report on CCG 1953 from the Children's Oncology Group. Blood. 2006 Jul 15;108(2):441-51.
9. Nagayama J, Tomizawa D, Koh K, Nagatoshi Y, Hotta N, Kishimoto T, et al. Infants with acute lymphoblastic leukemia and a germline MLL gene are highly curable with use of chemotherapy alone: results from the Japan Infant Leukemia Study Group. Blood. 2006 Jun 15;107(12):4663-5.
10. Barry E, DeAngelo DJ, Neuberg D, Stevenson K, Loh ML, Asselin BL, et al. Favorable outcome for adolescents with acute lymphoblastic leukemia treated on Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium Protocols. J Clin Oncol. 2007 Mar 1; 25(7):813-9.
11. Mattano LA, Jr., Sather HN, Trigg ME, Nachman JB. Osteonecrosis as a complication of treating acute lymphoblastic leukemia in children: a report from the Children's Cancer Group. J Clin Oncol. 2000; 18(18):3262-72.
12. Boissel N, Auclerc MF, Lheritier V, Perel Y, Thomas X, Leblanc T, et al. Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 trials. J Clin Oncol. 2003 Mar 1;21(5):774-80.
13. Stock W, La M, Sanford B, Bloomfield CD, Vardiman JW, Gaynon P, et al. What determines the outcomes for adolescents and young adults with acute lymphoblastic leukemia treated on cooperative group protocols? A comparison of Children's Cancer Group and Cancer and Leukemia Group B studies. Blood. 2008 Sep 1;112(5):1646-54.
14. Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grumayer R, Moricke A, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood. 2010 Apr 22;115(16):3206-14.
15. Moorman AV, Ensor HM, Richards SM, Chilton L, Schwab C, Kinsey SE, et al. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. Lancet Oncol. 2010 May;11(5):429-38.
16. Goldberg JM, Silverman LB, Levy DE, Dalton VK, Gelber RD, Lehmann L, et al. Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium experience. J Clin Oncol. 2003 Oct 1;21(19):3616-22.
17. Pui CH, Rubnitz JE, Hancock ML, Downing JR, Raimondi SC, Rivera GK, et al. Reappraisal of the clinical and biologic significance of myeloid-associated antigen expression in childhood acute lymphoblastic leukemia. J Clin Oncol. 1998 Dec;16(12):3768-73.
18. Uckun FM, Sather HN, Gaynon PS, Arthur DC, Trigg ME, Tubergen DG, et al. Clinical features and treatment outcome of children with myeloid antigen positive acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood. 1997;90(1):28-35.
19. Burger B, Zimmermann M, Mann G, Kuhl J, Loning L, Riehm H, et al. Diagnostic cerebrospinal fluid examination in children with acute lymphoblastic leukemia: significance of low leukocyte counts with blasts or traumatic lumbar puncture. J Clin Oncol. 2003 Jan 15;21(2):184-8.

20. Pui CH, Campana D, Pei D, Bowman WP, Sandlund JT, Kaste SC, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med. 2009 Jun 25;360(26):2730-41.

21. Gajjar A, Harrison PL, Sandlund JT, Rivera GK, Ribeiro RC, Rubnitz JE, et al. Traumatic lumbar puncture at diagnosis adversely affects outcome in childhood acute lymphoblastic leukemia. Blood. 2000 Nov 15;96(10):3381-4.

22. Sutcliffe MJ, Shuster JJ, Sather HN, Camitta BM, Pullen J, Schultz KR, et al. High concordance from independent studies by the Children's Cancer Group (CCG) and Pediatric Oncology Group (POG) associating favorable prognosis with combined trisomies 4, 10, and 17 in children with NCI Standard-Risk B-precursor Acute Lymphoblastic Leukemia: a Children's Oncology Group (COG) initiative. Leukemia. 2005 May;19(5):734-40.

23. Schultz KR, Pullen DJ, Sather HN, Shuster JJ, Devidas M, Borowitz MJ, et al. Risk- and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). Blood. 2007 Feb 1;109(3):926-35.

24. Attarbaschi A, Mann G, Konig M, Dworzak MN, Trebo MM, Muhlegger N, et al. Incidence and relevance of secondary chromosome abnormalities in childhood TEL/AML1+ acute lymphoblastic leukemia: an interphase FISH analysis. Leukemia. 2004 Oct;18(10):1611-6.

25. Nachman JB, Heerema NA, Sather H, Camitta B, Forestier E, Harrison CJ, et al. Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. Blood. 2007 Aug 15;110(4):1112-5.

26. Pui CH, Gaynon PS, Boyett JM, Chessells JM, Baruchel A, Kamps W, et al. Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet. 2002;359(9321):1909-15.

27. Arico M, Valsecchi MG, Camitta B, Schrappe M, Chessells J, Baruchel A, et al. Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med. 2000;342(14):998-1006.

28. Schultz KR, Bowman WP, Aledo A, Slayton WB, Sather H, Devidas M, et al. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol. 2009 Nov 1;27(31):5175-81.

29. Gaynon PS, Angiolillo AL, Carroll WL, Nachman JB, Trigg ME, Sather HN, et al. Long-term results of the children's cancer group studies for childhood acute lymphoblastic leukemia 1983-2002: a Children's Oncology Group Report. Leukemia. 2010 Feb;24(2):285-97.

30. Nachman JB, Sather HN, Sensel MG, Trigg ME, Cherlow JM, Lukens JN, et al. Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. N Engl J Med. 1998;338(23):1663-71.

31. Silverman LB, Gelber RD, Young ML, Dalton VK, Barr RD, Sallan SE. Induction failure in acute lymphoblastic leukemia of childhood. Cancer. 1999;85(6):1395-404.

32. Oudot C, Auclerc MF, Levy V, Porcher R, Piguet C, Perel Y, et al. Prognostic factors for leukemic induction failure in children with acute lymphoblastic leukemia and outcome after salvage therapy: the FRALLE 93 study. J Clin Oncol. 2008 Mar 20;26(9):1496-503.

33. Balduzzi A, Valsecchi MG, Uderzo C, De Lorenzo P, Klingebiel T, Peters C, et al. Chemotherapy versus allogeneic transplantation for very-high-risk childhood acute lymphoblastic leukaemia in first complete remission: comparison by genetic randomisation in an international prospective study. Lancet. 2005 Aug 20-26;366(9486):635-42.

34. Coustan-Smith E, Behm FG, Sanchez J, Boyett JM, Hancock ML, Raimondi SC, et al. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet. 1998 Feb 21;351(9102):550-4.

35. Zhou J, Goldwasser MA, Li A, Dahlberg SE, Neuberg D, Wang H, et al. Quantitative analysis of minimal residual disease predicts relapse in children with B-lineage acute lymphoblastic leukemia in DFCI ALL Consortium Protocol 95-01. Blood. 2007 Sep 1;110(5):1607-11.

36. Borowitz MJ, Devidas M, Hunger SP, Bowman WP, Carroll AJ, Carroll WL, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood. 2008 Jun 15;111(12):5477-85.

37. Coustan-Smith E, Sancho J, Behm FG, Hancock ML, Razzouk BI, Ribeiro RC, et al. Prognostic importance of measuring early clearance of leukemic cells by flow cytometry in childhood acute lymphoblastic leukemia. Blood. 2002 Jul 1;100(1):52-8.

38. Sirvent N, Suciu S, Bertrand Y, Uyttebroeck A, Lescoeur B, Otten J. Overt testicular disease (OTD) at diagnosis is not associated with a poor prognosis in childhood acute lymphoblastic leukemia: Results of the EORTC CLG study 58881. Pediatr Blood Cancer. 2007 Sep;49(3):344-8.

39. Hijiya N, Liu W, Sandlund JT, Jeha S, Razzouk BI, Ribeiro RC, et al. Overt testicular disease at diagnosis of childhood acute lymphoblastic leukemia: lack of therapeutic role of local irradiation. Leukemia. 2005 Aug;19(8):1399-403.

40. Crist WM, Carroll AJ, Shuster JJ, Behm FG, Whitehead M, Vietti TJ, et al. Poor prognosis of children with pre-B acute lymphoblastic leukemia is associated with the t(1;19)(q23;p13): a Pediatric Oncology Group study. Blood. 1990;76(1):117-22.

41. Moorman AV, Richards SM, Robinson HM, Strefford JC, Gibson BE, Kinsey SE, et al. Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). Blood. 2007 Mar 15;109(6):2327-30.

Current Treatment Approaches in Childhood Acute Lymphoblastic Leukemia

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Abstract

Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood and has served as a model system for clinical and basic research beyond pediatric hemato-oncology since the early 1960s. Nowadays, as a result of these prolonged and well-organized research efforts, childhood ALL can be successfully treated in about 80% of patients by the application of intensive combination chemotherapy regimens, which in specific patient subgroups may need to be supplemented with radiation therapy and/or hematopoietic stem cell transplantation. Triggered through the observation of several clinical presenting features, biological characteristics and early treatment response being associated with treatment outcome, therapy intensity on contemporary ALL protocols is adjusted according to prognostic factors predicting the risk of ALL relapse. However, although the goal of developing effective therapy for the majority of children with ALL has been achieved, significant numbers of patients still die due to recurrent disease or the toxicity of treatment applied. Thus, future research activities will have to improve our molecular understanding of leukemia and host factors underlying the differences in treatment response and outcome and to finally address the therapeutic needs of the individual child.

Introduction

Acute lymphoblastic leukemia (ALL) represents the malignant proliferation of lymphoid cells blocked at early stages of differentiation and is the most common malignancy in children (1). It accounts for approximately 25% of all childhood cancers and about 80% of childhood leukemias (1,2). The annual incidence rate of childhood ALL varies world-wide between approximately one and four new cases per 100,000 children younger than 15 years, with a peak incidence at approximately two to five years of age (2-6). More affluent countries tend to have higher incidence rates (2,3). However, incidence rates for childhood ALL vary not only between countries, but also by ethnicity within countries: in the US Hispanic children have the highest incidence and the rate is higher in white as compared to that in black children (4). More than 60% of patients diagnosed with ALL are children (1,2).

Treatment results in childhood ALL are one of the true success stories of clinical oncology with current overall cure rates of approximately 80% in developed countries (Table 1, Figure 1) (7-21).

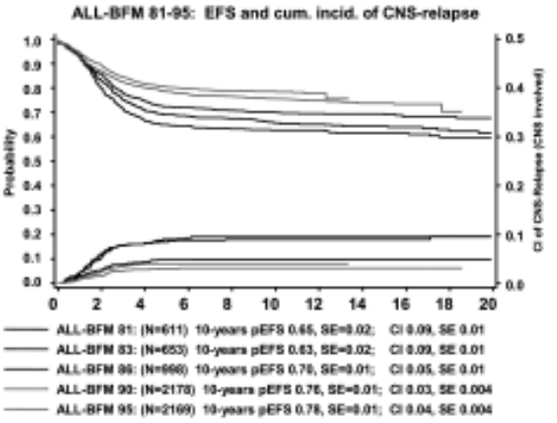

Figure 1.

Event-free survival curves for patients treated on five consecutive ALL-BFM trials from 1981 to 2000.

These results are reached by application of intensive multiagent chemotherapeutic regimens and in specific patient subgroups additional radiotherapy and/or hematopoietic stem cell transplantation (HSCT). Modern treatment regimens consist of at least four phases: (i) an induction period aiming at an initial remission induction within approximately 4 to 6 weeks through the use of multiple cancer

chemotherapeutic drugs; (ii) consolidation/ intensification and reinduction segments to eradicate residual leukemic blasts in patients who are in remission by morphologic criteria; (iii) extracompartment therapy such as central nervous system (CNS) preventive therapy, and (iv) a maintenance period to further stabilize remission by suppressing re-emergence of a drug-resistant clone through continuing reduction of residual leukemic cells (22,23). As certain clinically and biologically distinct patient subgroups with ALL have a particular poor outcome on standard ALL treatment, clinical protocols specifically addressing the potential therapeutic needs of these subgroups have been initiated in the recent past (e.g., hybrid protocols for infants, and imatinib-including regimens for BCR/ABL-positive ALL) (24,25).

Table 1: List of study groups which have recently reported long-term treatment results

Study group	Period of enrollment	Age group eligible	No. of pts	No. of studies	Event-free survival at 10y[+]	Ref.
AIEOP	1982-2000	≤ 15 y *	4865	5	71.7 ± 1.3%	7
BFM	1981-2000	< 18 y	6609	5	78.0 ± 1.1%	8
CCG	1983-2002	< 21 y	13298	16	72.6 ± 2.9%	9
COALL	1982-2003	< 18 y	1967	5	76.3 ± 3.0%	10
CPH	1990-2002	< 18 y	730	2	72.1 ± 2.3%	11
DCOG	1984-2004	< 18 y	1734	4	70.0 ± 2.1%	12
DFCI	1985-2000	< 18 y	1457	4	80.8 ± 2.1%	13
INS	1984-2003	< 18 y	786	3	76.5 ± 2.4%[++]	14
JCCLSG	1981-1993	< 18 y	1021	4	63.4 ± 3.3%[#]	15
NOPHO	1992-2007	1 - < 15 y	2668	2	75.0 ± 1.0%	16
POG	1984-2001	1 - < 22 y	7393	12	73.2 ± 2.1%[§]	17
SJCRH	1984-1999	≤ 18 y	1011	5	77.6 ± 2.9%	18
TCCSG	1984-1995	1 - < 15 y	1846	4	75.0 ± 1.8%	19
TPOG	1997-2007	≤ 18 y	1390	2	72.5 ± 1.3%	20
UK-WPCL	1980-2002	≤ 15 y	6516	4	74.1 ± 1.0%	21

[+] listed here are the best results reported by each study group; * < 18 y in trial AIEOP-95; [++] at 8 years; [#] at 12 years; [§] only in B-lineage (10y-EFS in T-ALL was 72.2 ± 4.7%);

AIEOP: Associazione Italiana di Ematologia ed Oncologia Pediatrica (Italy); BFM: Berlin-Frankfurt-Münster ALL Study Group (Germany, Austria, Switzerland); CCG: Children's Cancer Group (USA); COALL: Cooperative ALL Study Group (Germany); DCOG: Dutch Childhood Oncology Group (Netherlands); DFCI: Dana-Farber Cancer Institute ALL Consortium (USA); INS: Israeli National Studies of childhood ALL; JCCLSG: Japanese Childhood Cancer and Leukemia Study Group; NOPHO: Nordic Society of Pediatric Hematology and Oncology; POG: Pediatric Oncology Group (USA); SJCRH: St. Jude Children's Research Hospital (USA); TCCSG: Tokyo Children's Cancer Study Group; TPOG: Taiwan Pediatric Oncology Group; UKALL: UK Medical Research Council Working Party on Childhood Leukaemia (U.K.).

Clinical presentation and diagnosis

The initial clinical presentation of a child with ALL largely depends on the extent of the leukemic infiltration of the bone marrow and extramedullary sites. Typical clinical signs are fever, pallor, fatigue, bruises, enlargement of liver, spleen and lymph nodes, and pain (e.g., bone pain). In most patients, complete blood cell counts show anemia, thrombocytopenia and granulocytopenia with or without concomitant leukocytosis. The diagnosis of ALL is usually made by cytomorphological and cytochemical examination of a bone marrow aspirate and in difficult cases by Jamshidi needle biopsy and is established when at least 25% lymphoblasts are present in the marrow (27). CNS involvement (CNS3 status) is diagnosed by the presence of blasts in the cerebrospinal fluid (CSF; for definition see Table 2) or if intracerebral infiltrates are detected by cross-sectional radiological imaging (28). Initial diagnostics are complemented by flow cytometry-based immunophenotyping to gain information on the blasts expression of lymphoid differentiation-

associated antigens as measured by the reactivity to specific monoclonal antibodies and to determine the cellular DNA content of leukemic cells (29, 30). In addition, a combined approach using cytogenetic and molecular genetic techniques is used for the detection of genetic aberrations, such as non-random recurrent chromosomal translocations or their molecular equivalents (e.g., the t (9;22) or the BCR/ABL fusion transcript) (31-37). Molecular-genetic techniques and/or flow cytometry are also used to monitor disease burden during therapy by measuring minimal residual disease (MRD) (38-49). A last important issue addresses the definition of what is called complete remission and relapse: complete remission is defined as the absence of leukemic blasts in blood and CSF, fewer than 5% lymphoblasts in bone marrow aspiration smears, and no evidence of localized disease. Relapse is defined as the recurrence of lymphoblasts or localized leukemic infiltrates at any site. The new MRD detection methods have required a more detailed review of these definitions (50).

Prognostic factors and risk-adapted treatment

Continuing research on the clinical and biological aspects of ALL has identified numerous features with prognostic potential some of which are displayed in Table 2 (26, 28, 30-67). On modern protocols, risk-adapted therapy reflecting the probability of treatment failure has become a common feature in the clinical management of childhood ALL. For this purpose, the initially assessed prognostic factors are used to estimate an individual patient's risk of relapse and to adjust the required treatment intensity by therapy stratification into different risk groups (e.g., standard/low, intermediate, high) (1,7-21).

Table 2. Important prognostic factors[a] and their approximate incidences in childhood ALL.

Factor	Favorable prognostic factors and their approximate incidence (%)	Unfavorable or less favorable prognostic factors and their approximate incidence (%)
Age at diagnosis	≥ 1 and < 10 years (77%)	< 1 year (3%) or ≥ 10 years (20%)
Gender	female (45%)	male (55%)
White blood cell count at diagnosis	< 50.000/µl (80%)	≥ 50.000/µl (20%)
Immunophenotype	CD10-positive precursor B-cell ALL (83%)	CD10-negative precursor B-cell ALL (4%), T-ALL (13%)
CNS disease[b]	CNS 1 (80%)	CNS 3 (3%), TLP+ (7%)
Genetic features[c]	hyperdiploidy (20%), TEL/AML1 positivity (20%)	hypodiploidy (1%), t(9;22) or BCR/ABL positivity (2%), t(4;11) or MLL/AF4 positivity (2%)
Prednisone response[d]	< 1000/µl blood blasts (90%)	≥ 1000/µl blood blasts (10%)
Early bone marrow response	< 5% blasts (M1) on day 15 of induction treatment (60%)	≥ 25% blasts (M3) on day 15 of induction treatment (15%)
Remission status after induction therapy in the bone marrow (morphologically assessed)	< 5% blasts (M1) after 4 to 5 weeks of induction treatment (98%)	≥ 5% blasts (M2 or M3) after 4 to 5 weeks of induction therapy (2%)
Minimal residual disease[e] in the bone marrow (molecularly assessed)	< 10^{-4} blasts after 5 weeks of induction treatment (40%)	≥ 10^{-3} blasts after 12 weeks of treatment (induction and consolidation) (10%)

[a] *prognostic factors are treatment dependent and, therefore, the selection presented in the table above cannot be entirely comprehensive; it reflects the current recommendations of the German BFM study group.*

[b] *CNS1 (puncture nontraumatic, no leukemic blasts in the cerebrospinal fluid (CSF) after cytocentrifugation); CNS3 (puncture nontraumatic, >5 leukocytes/µL CSF with identifiable blasts); TLP+ (traumatic lumbar puncture with identifiable leukemic blasts); a TLP with no identifiable blasts is not an adverse factor; the prognostic impact of CNS2 status (puncture nontraumatic, ≥5 leukocytes/µL CSF with identifiable blasts) is debated. For cytomorphological examination, CSF samples should be analyzed after cytospin preparation, a method through which cellular components within the CSF are concentrated by centrifugation.*

[c] *hyperdiploidy defined as the presence of more than 50 chromosomes or a DNA index (the ratio of DNA content in leukemic G0/G1 cells to that of normal diploid lymphocytes) ≥1.16; hypodiploidy defined by <45 chromosomes; the prognostic value of MLL gene rearrangements other than MLL/AF4 and presence of the E2A/PBX1 fusion transcript are debated.*

[d] *after 7 days induction with daily prednisone and a single intrathecal dose of methotrexate on treatment day 1.*

[e] *assessed by molecular genetic techniques or flow cytometry; markers required to have a sensitivity of at least 10^{-4}.*

The prognostic significance of an inadequate early reduction of leukemic blasts in the peripheral blood was first described by the BFM study group and confirmed by several other study groups (62, 65, 68). Of importance, the specificity of response evaluation might vary with the composition of the induction regimen and the time point of response evaluation (63, 64, 66, 67). Although a poor early response to induction therapy as described above is highly predictive of treatment failure, the majority of recurrences occurs in the large group of patients with an adequate morphological response to treatment. Of advantage in this context, the sub-microscopic assesssment of MRD is approximately 1,000 to 10,000-fold more sensitive compared to methods based on morphological detection and provides excellent prognostic information (38-50). Although most of the experience on MRD in clinical settings was gained through DNA-PCR-based detection of leukemic clone-specific immunoglobulin and/or T-cell receptor gene rearrangements, flow-cytometry-based analyses by detection of specific antigen patterns of the leukemic clone also produced sensitive and reliable results comparable to PCR-based methods (41-43, 46, 47).

Remission induction

Contemporary treatment approaches for childhood ALL aim at an initial remission induction to restore normal hematopoiesis within approximately 4 to 6 weeks. In most study groups this goal is achieved in approximately 98% of patients through the systemic application of three drugs (glucocorticoid, vincristine, L-asparaginase) to which an anthracycline may be added as a fourth drug (1, 7-21). On ALL-BFM protocols, remission induction is initiated by a 7-day monotherapy with orally administered prednisone (and one intrathecal dose of intrathecal methotrexate on day 1), which is particularly useful in avoiding complications related to extensive tumor cell lysis. Undoubtedly, the dose intensity of the induction phase can have a major impact on the overall treatment outcome (1, 26, 69, 70, 76). Nevertheless, in specific subgroups of childhood ALL, the necessity of a four-drug induction regimen is subject to debate and it is, for example, unclear if addition of an anthracycline to a three-drug induction regimen is of real benefit to certain low-or intermediate-risk patients (71-73). The clinical anti-leukemic benefit of effective asparagine depletion in induction has been demonstrated (74).

Another frequently discussed issue addresses the choice of the glucocorticoid for optimal induction. Despite some debate on a truly equivalent dose, compared to prednisolone, dexamethasone appears to have a stronger antileukemic effect in vitro and has been shown to provide better leukemic CNS control and lower relapse rates (75-83). However, dexamethasone was also associated with increased side-effects including severe infectious complications (81-83). Table 3 summarizes some of the experiences gained through studies comparing prednisolone with dexamethasone in the treatment of childhood ALL. A comparison of dexamethasone at 10 mg/m^2/d vs. 60 mg/m^2/d prednisolone in induction is currently evaluated in a large international trial (47-49).

The 2% of patients not in remission after induction therapy will either have died of treatment- or disease-related complications or display nonresponsive disease. The latter group includes patients that will achieve only delayed remission or show resistant disease. Because of the poor prognosis of this minor non-responsive patient population, alternative therapeutic approaches should be considered early during the disease process (84, 85).

Table 3. Selected studies comparing prednisolone (pred) with dexamethasone (dexa) in the treatment of childhood ALL.

Trial	Years	Study design	Study population	Glucocorticoid dose[a]	Dexa vs. pred administration in induction	Dexa vs. pred administration elsewhere	Outcome[b]	Ref.
CALGB 7111	1971-1974	randomized	all risk groups (n=646)	pred 40 mg/m2/d vs. dexa 6 mg/m2/d	[c] days 1-28 or days 1-21 or days 11-31	7-day pulses in maintenance	Isolated CNS relapse rates: pred 25.5%; dexa 14.3%	76
DCLSG ALL VI	1984-1988	historical control	non-HR patients[d] (n=190)	dexa 6 mg/m2/d Historical control DCLSG ALL V (n=240) had similar eligibility criteria and used pred 40 mg/m2/d ininduction, less intrathecal therapy and no medium high-dose methotrexate (2g/m2), but cranial irradiation at age-dependent doses of 15 to 25 Gy.	days 1-28	14-day pulses in maintenance	EFS at 10 years on DCLSG ALL VI (82±3) was almost 30% better compared to ALL V Isolated CNS relapse rates: ALL VI 1.1%; ALL V 12.9%	77
DFCI 91-01	1991-1995	historical control	all risk groups (n=377)	SR patients: dexa 6 mg/m2/d HR patients: dexa 18 mg/m2/d Historical control DFCI 87-01 used pred 40 mg/m2/d in SR and pred 120 mg/m2/d in HR patients, less asparaginase in intensification (20 instead of 30 weeks), and not all patients received high-dose methotrexate as it was randomized to low-dose.	–	5-day pulses in intensification and maintenance	EFS at 5 years DFCI 91-01 83±2%; DFCI 87-01 78±2% Isolated CNS relapse rates: DFCI 91-01 1.1%; DFCI 87-01 4.1%	78
CCG 1922	1993-1995	randomized	SR patients[e] (n=1060)	pred 40 mg/m2/d vs. dexa 6 mg/m2/d -	days 1-28	5-day pulses in consolidation and maintenance	EFS at 6 years pred 77±2%; dexa 85±2% Isolated CNS relapse rates: pred 7.1%; dexa 3.7% Excess of myopathy and hypergly cemia with dexa; behavioural problems and tendency towards pancreatic toxicity with dexa.	79
UK MRC ALL97 and ALL97/99	1997-2002	randomized	all risk groups (n=1603)	pred 40 mg/m2/d vs. dexa 6.5 mg/m2/d	days 1-28 in ALL97 and days 1-29 in ALL97/99	5-day pulses in interim maintenance and maintenance	EFS at 5 years pred 76±3%; dexa 84±3% Isolated CNS relapse rates: pred 5.0%; dexa 2.5% Excess of behavioural problems, myopathy, osteopenia and weight gain with dexa.	81
TCCSG L95-14	1995-1999	randomized	only SR and IR patients[f] (n=359)	pred 60 mg/m2/d vs. dexa 8 mg/m2/d; during intensification reduced to: pred 40 mg/m2/d vs. dexa 6 mg/m2/d	days 1-31	days 1-14 in four intensification elements for SR and three elements for IR patients	No differences in EFS at 8 years[g]; no statistically significant difference with regard to site of relapse or toxicity; a tendency towards less CNS relapses with dexa was set off by an increase in bone marrow relapses in SR; tendency towards higher incidence of complications with dexa.	80

[a] taper not indicated.; [b] EFS = event-free survival

[c] depending on asparaginase randomization in induction (no asparaginase: days 1-28; 10 days asparaginase before (days 11-31), concurrent with or after glucocorticoid and vincristine induction (days 1-21).

[d] age 0 to 15 years, initial white blood cell counts lower than 50.000/µl, and absence of a mediastinal mass and/or cerebromeningeal leukemia at diagnosis, no B-ALL.

[e] age 1 to less than 10 years with white blood cell counts lower than 50.000/µl, no lymphoma syndrome, no B-ALL.

[f] SR: non-T phenotype, age 1 to 6 years, white blood cell count less than 20.000/µl at diagnosis; IR: age 1 to 6 years and a white blood cell count between 20 and 100.000/µl, age 7 to 9 years, white blood cell count less than 20.000/µl; or a definition of SR with T-cell markers. Patients positive cytogenetics for a t(9;22), 11q23 aberrations or a t(1;19), a mediastinal mass or meningeal infiltration were excluded from both groups and stratified into the HR group.

[g] SR pred 84±5%; SR dexa 81±4%; IR pred 80±5%; IR dexa 85±5%.

Consolidation/intensification and reinduction treatment

Eradication of residual leukemic blasts in patients who are in remission by morphologic criteria is the primary aim of consolidation/ intensification treatment. Consolidation/ intensification treatment is necessary as patients successfully induced into remission, but not given additional treatment, usually relapse within months. A so-called reinduction or delayed intensification treatment can further enhance the effect of previous consolidation/intensification therapy both in low and high risk patients (86-88). The consolidation/intensification phases administered in protocols of the large study groups on treatment of childhood ALL may differ, for example, with regard to amounts, timing, and number of drug doses, drug composition and overall treatment context. Thus, the direct impact of most of these consolidation/intensification strategies and/or their individual components is difficult to assess. Today, most protocols use high-dose methotrexate (combined with folinic acid rescue) together with 6-mercaptopurine (6-MP) and/or prolonged administrations of asparaginase in consolidation/intensification (7-21, 69, 70, 89, 90). Reinduction treatment mainly consists of a late repetition of the initial remission induction and early intensification phases (71, 88). A randomized trial by the Children's Cancer Group applying an augmented BFM protocol showed that intensified consolidation and double-delayed intensification can further improve the outcome of high-risk patients with a slow initial treatment response (91). Of interest, a recent subsequent trial on higher-risk patients with a rapid marrow response to induction therapy by the same group, demonstrated an improved event-free survival with more intensive but not with longer postinduction intensification treatment (92). Unfortunately, further intensification of treatment including higher doses of glucocorticoids have been associated with a high incidence of osteonecrosis, especially in older children (93). Consequently, some investigators suggest glucocorticoid administriation in intensification/ consolidation on alternate weeks for children older than 10 years to reduce the complication rates (92).

Central nervous system-directed therapy

CNS-directed therapy has become a prerequisite for successful treatment of childhood ALL. Before its introduction in the 1960s, more than 50% of children with ALL suffered from disease recurrence originating in the CNS (1, 22). This high rate could be reduced to less than 5% through the introduction of cranial irradiation, intrathecal chemotherapy with methotrexate alone or in combination with other drugs (cytarabine, hydrocortisone), and systemic application of chemotherapeutics with adequate penetration into the CNS (high-dose methotrexate, dexamethasone, high-dose cytarabine) (1,7-21). The intensity of CNS-directed treatment is adjusted according to the risk of ALL relapse in the CNS, the most important risk factor being overt CNS involvement at diagnosis (CNS3) (28, 99-101). Additional risk factors include a high initial white blood cell count, pro-B or precursor T-cell immunophenotype, t (9;22) or t (4;11), and a traumatic lumbar puncture with identifiable blast cells present at diagnosis (66, 101). CNS-directed therapy may differ in the number of intrathecal injections and/or intrathecally applied drugs, as well as in the inclusion of cranial irradiation at different doses (7-21, 94-96). Excluding infants, most clinical protocols administering intensive systemic therapy still recommend preventive cranial irradiation (12 or 18 Gy) for high-risk patients and/or those with a precursor T-cell immunophenotype - at least for those with white blood cell counts of 100.000/μl or more at diagnosis. In T-ALL with high WBC, elimination of preventive cranial radiation has caused a significant increase of systemic recurrences (95). Patients with CNS2 status or a traumatic lumbar puncture are recommended to receive additional therapeutic doses of intrathecal chemotherapy. Also CNS3 patients receive more intense intrathecal chemotherapy and, in addition, are subject to therapeutic cranial irradiation (18 or 24 Gy when ^{3} two years of age; younger children should receive reduced

doses). All other patients (precursor B-cell ALL, CNS1, non HR) should receive preventive intrathecal chemotherapy. More recently, the best-balanced strategy for CNS prophylaxis in ALL treatment has been debated (97, 98). New molecular marker may define the risk of CNS involvement and recurrence more precisely than the blast count in the CSF (102).

Allogeneic hematopoietic stem cell transplantation

Results of frontline and relapse protocols have improved over time. At the same time, the experience gained also led to advances in HSCT procedures. The continuous parallel developments in both fields complicate the description of the exact role of HSCT in childhood ALL and elucidate the strong need for prospective clinical trials (103). In 2003, the ALL-BFM and the ALL-REZ BFM study groups initiated a prospective, international, multicenter trial (ALL-SCT-BFM 2003) which will now be extended to a larger international consortium (104). This trial exactly defined procedures on HLA-typing, donor selection, conditioning regimen, graft versus host disease prophylaxis and therapy as well as standards of supportive care ensure a high degree of standardization with regard to all relevant components potentially associated with the heterogeneity in outcome observed in the context of HSCT. It is expected that the results of such prospective trials will more precisely determine the indication of the different HSCT procedures in high-risk or relapsed childhood ALL. Meanwhile, HSCT in children with ALL in first remission should be confined to patients whose disease is associated with poor prognostic features such as the t (9;22) or a poor response to remission induction therapy (105-107).

Maintenance therapy

Hypothetically, maintenance treatment aims at a further stabilization of remission by suppressing the re-emergence of a drug-resistant clone through consistently reducing the pool of residual leukemic cells. The current standard of maintenance therapy consists of up to two or three years of treatment (from initial time of diagnosis) with daily oral 6-MP and weekly oral methotrexate (7-21). The combination of 6-MP with methotrexate acts synergistically as methotrexate inhibits purine de novo synthesis, leading to a higher intracellular availability and increased incorporation of phosphorylated thiopurines in DNA and RNA (108-110). During maintenance treatment, 6-MP and methotrexate doses are adjusted according to absolute leukocyte or neutrophil and platelet counts. Important to note and a potential source of heterogeneity with regard to outcome analyses, the starting dose as well as dose adjustment guidelines while on therapy may differ between the different study groups. As several reports suggested an improved outcome with bedtime administration, 6-MP is commonly administered in the evening hours (110, 111). Also, 6-MP should not be given in combination with milk since the xanthine oxidase activity contained in milk decreases the bioavailability of 6-MP (112). Of utmost clinical importance, at St Jude Childrens' Research Hospital researchers have demonstrated that maintaining the highest tolerable dose of daily 6-MP in maintenance therapy is an important prognostic factor in childhood ALL (113). Intensification of maintenance treatment by the administration of vincristine/dexamethasone pulses was recently shown to provide no extra benefit (114). The reduction of maintenance therapy to less than 2 years (from the time point of initial diagnosis) was associated with an increased frequency of leukemic relapses (115). Although it was proven disadvantageous to shorten maintenance treatment, whether or not extended maintenance of up to 3 years is offering any beneficial effect for particular subgroups in the context of different treatment strategies is not completely evaluated. With regard to the debate on the better thiopurine, three randomized studies compared the toxicity and efficacy of 6-thioguanine with 6-MP in interim maintenance and maintenance therapy of childhood ALL (Table 4) (116-118). However, due to the observation of dose-dependent high rates of severe hepatotoxic side-effects associated with the application of 6-thioguanine, the current thiopurine drug of choice for maintenance treatment remains 6-MP.

Table 4. Randomized trials of 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG) for childhood ALL.

Trial	Years	Study population	Thiopurine randomization	Outcome[a]	Observed Toxicity	Ref.
COALL-92	1992-1997	all risk groups (n=474)	6-MP 75 mg/m2/d vs. 6-TG 50/40 mg/m2/d	EFS at 6.6 years 6-MP 79%; 6-TG 78%	Prolonged myelosuppression with marked thrombocytopenia under 6-TG	116
UK MRC ALL97 and ALL97/99	1997-2002	all risk groups (n=1498)	6-MP 75 mg/m2/d vs. 6-TG 40 mg/m2/d	EFS at 6.6 years 6-MP 81%; 6-TG 80%	11% of patients in the 6-TG arm developed non-fatal hepatic toxicity with features of veno-occlusive disease; a lower risk of isolated CNS relapse with 6-TG was offset by an increased risk of death in remission mainly due to infections during maintenance.	117
CCG-1952	1996-2000	only SR patients[b] (n=2026)	6-MP 75 mg/m2/d vs. 6-TG 60/50 mg/m2/d	EFS at 5 years 6-MP 77%; 6-TG 85%	20% of patients in the 6-TG arm developed reversible veno-occlusive disease; 6-TG was associated with portal hypertension as a late-effect.	118

[a] EFS = event-free survival

[b] age 1 to less than 10 years with white blood cell counts lower than 50.000/μl, no lymphoma syndrome, no B-ALL

Relapse

During the last decades, prospective attempts on the treatment of children with relapsed ALL have been conducted (119-124). Similarly to frontline ALL therapy, treatment outcome after first relapse depends on clinical and biological characteristics of the leukemia. A short duration of first clinical remission, bone marrow involvement, a precursor T-cell immunophenotype, and unfavorable chromosomal aberrations [e.g., a t (9;22)] have been identified as the most important poor prognostic factors at time of relapse of ALL. In addition, MRD levels during the initial course of relapse treatment were shown to be of prognostic value (125). Roughly, conventional intensive chemotherapy and radiotherapy can cure up to one third of children with relapsed ALL, with percentages ranging from 0 to 70% depending on the pattern of prognostic factors present at relapse. For patients with early systemic relapse (within 18 months of achieving first complete remission) , HSCT from an HLA-identical sibling is currently thought to be the treatment of choice. In the situation of a HSCT from an unrelated donor, due to potentially higher toxicity, beneficial effects may be restricted to high-risk patients. For other subgroups of relapsed ALL (e.g., late relapses, extramedullary disease) , the role of allogeneic HSCT remains controversial and prospective trials are needed (103, 104).

Late effects of treatment

Quality of treatment has become more important since the major study groups have reached relatively comparable rates of long-term event-free survival. Unfortunately, with overall improvements in survival, the long-term adverse effects of treatment have become apparent, as well. These include cardiac late effects (anthracycline therapy-associated cardiomyopathy), neuropsychologic (e.g., methotrexate therapy-associated) and endocrinologic deficits, as well as secondary neoplasms such as acute myeloid leukemia associated with topoisomerase II inhibitor treatment and brain tumors associated with radiotherapy (126-128). The long-term adverse effects differ according to many factors including individual's health status and the treatment received. Therefore, it is important that leukemia survivors receive regular exams by health care professionals who are familiar with leukemia treatment and the associated risks and who are able to recognize the early signs of late effects. Meanwhile, some study groups provide extensive recommendations for screening and

management of late effects after treatment for childhood ALL (128). These long-term follow-up approaches will not only improve the health and quality of life for survivors, but also provide an improved infrastructure for systematic studies on long-term consequences of childhood ALL treatment and, hopefully, their future prevention.

Perspective

Conventional methods of risk classification in childhood ALL including standard MRD analyses provide excellent tools for clinical treatment stratification of childhood ALL. In addition, MRD analysis for "phenotypic" characterization offers the ability to molecularly discern clinically relevant differences that may be of importance for developing a better understanding of leukemias and advancing therapeutic strategies. Thus, MRD analysis in combination with a comprehensive evaluation of leukemia and host characteristics holds the potential to further improve treatment by leading to an even more exact and earlier characterization of patients at true risk of relapse. Both comprehensive molecular characterization and early identification of these patients will be essential in future clinical trials in order to utilize the optimal therapy in the first treatment cycles and, for those in need of it, to secure the timely introduction of potential targeted treatment based on individual molecular characteristics of leukemic cells. Of importance, all future approaches should be evaluated in close context with "classical" risk-adapted treatment strategies and molecular monitoring of treatment response.

References

1. Pui CH, Evans WE: Treatment of childhood acute lymphoblastic leukemia. N Engl J Med 354:166-178, 2006
2. Redaelli A, Laskin BL, Stephens JM, Botteman MF, Pashos CL: A systematic literature review of the clinical and epidemiological burden of acute lymphoblastic leukaemia (ALL). Eur J Cancer Care (Engl) 14:53-62, 2005
3. Howard SC, Metzger ML, Wilimas JA, Quintana Y, Pui CH, Robison LL, et al: Childhood cancer epidemiology in low-income countries. Cancer 112:461-472, 2008
4. Linabery AM, Ross JA: Trends in childhood cancer incidence in the U.S. (1992-2004). Cancer 112:416-432, 2008
5. Spix C, Eletr D, Blettner M, Kaatsch P: Temporal trends in the incidence rate of childhood cancer in Germany 1987-2004. Int J Cancer 122::1859-1867, 2008
6. Swaminathan R, Rama R, Shanta V: Childhood cancers in Chennai, India, 1990-2001: incidence and survival. Int J Cancer 122:2607-2611, 2008
7. Conter V, Arico M, Basso G, Biondi A, Barisone E, Messina C, et al. Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) Studies 82, 87, 88, 91 and 95 for childhood acute lymphoblastic leukemia. Leukemia 24:255-64, 2010
8. Möricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, Gadner H, et al: Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia. 24 (2) :265-84, 2010.
9. Gaynon PS, Angiolillo AL, Carroll WL, Nachman JB, Trigg ME, Sather HN, et al. Long-term results of the children's cancer group studies for childhood acute lymphoblastic leukemia 1983-2002: a Children's Oncology Group Report. Leukemia 24:285-97, 2010
10. Escherich G, Horstmann MA, Zimmermann M, Janka-Schaub GE. Cooperative study group for childhood acute lymphoblastic leukaemia (COALL) : long-term results of trials 82, 85, 89, 92 and 97. Leukemia 24:298-308, 2010
11. Stary J, Jabali Y, Trka J, Hrusak O, Gajdos P, Hrstkova H, et al. Long-term results of treatment of childhood acute lymphoblastic leukemia in the Czech Republic. Leukemia 24:425-8, 2010
12. Kamps WA, van der Pal-de Bruin KM, Veerman AJ, Fiocco M, Bierings M, Pieters R. Long-term results of Dutch Childhood Oncology Group studies for children with acute lymphoblastic leukemia from 1984 to 2004. Leukemia 24:309-19, 2010
13. Silverman LB, Stevenson KE, O'Brien JE, Asselin BL, Barr RD, Clavell L, et al. Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985-2000). Leukemia 24:320-34, 2010
14. Stark B, Nirel R, Avrahami G, Abramov A, Attias D, Ballin A, et al. Long-term results of the Israeli National Studies in childhood acute lymphoblastic leukemia: INS 84, 89 and 98. Leukemia 24:419-24, 2010
15. Tsurusawa M, Shimomura Y, Asami K, Kikuta A, Watanabe A, Horikoshi Y, et al. Long-term results of the Japanese Childhood Cancer and Leukemia Study Group studies 811, 841, 874 and 911 on childhood acute lymphoblastic leukemia. Leukemia 24:335-44, 2010
16. Schmiegelow K, Forestier E, Hellebostad M, Heyman M, Kristinsson J, Soderhall S, et al. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia 24:345-54, 2010
17. Salzer WL, Devidas M, Carroll WL, Winick N, Pullen J, Hunger SP, et al. Long-term results of the pediatric oncology group studies for childhood acute lymphoblastic leukemia 1984-2001: a report from the children's oncology group. Leukemia 24:355-70, 2010
18. Pui CH, Pei D, Sandlund JT, Ribeiro RC, Rubnitz JE,

Raimondi SC, et al. Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. Leukemia 24:371-82, 2010

19. Tsuchida M, Ohara A, Manabe A, Kumagai M, Shimada H, Kikuchi A, et al. Long-term results of Tokyo Children's Cancer Study Group trials for childhood acute lymphoblastic leukemia, 1984-1999. Leukemia 24:383-96, 2010

20. Liang DC, Yang CP, Lin DT, Hung IJ, Lin KH, Chen JS, et al. Long-term results of Taiwan Pediatric Oncology Group studies 1997 and 2002 for childhood acute lymphoblastic leukemia. Leukemia. 2010 Feb; 24 (2):397-405

21. Mitchell C, Richards S, Harrison CJ, Eden T. Long-term follow-up of the United Kingdom medical research council protocols for childhood acute lymphoblastic leukaemia, 1980-2001. Leukemia 24:406-18, 2010

22. Pinkel, D: Five year follow-up of "Total Therapy" of childhood lymphocytic leukemia. JAMA 216:648-652, 1971

23. Riehm H, Gadner H, Henze G, Langermann H-J, Odenwald E: The Berlin childhood acute lymphoblastic leukemia therapy study, 1970-1976. Am J Pediatr Hematol Oncol 2:299-306, 1980

24. Pieters R, Schrappe M, De Lorenzo P, Hann I, De Rossi G, Felice M, et al: A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99) : an observational study and a multicentre randomised trial. Lancet 370:240-250, 2007

25. Schultz KR, Bowman WP, Aledo A, Slayton WB, Sather H, Devidas M, et al. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol 27:5175-81, 2009

26. Möricke A, Reiter A, Zimmermann M, Gadner H, Stanulla M, Dördelmann M, et al: Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood 111:4477-4489, 2008

27. Bennett JM, Catovski D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al: Proposals for the classification of the acute leukaemias. French-American-British (FAB) cooperative group. Br J Haematol 33:451-458, 1976

28. Mahmoud HH, Rivera GK, Hancock ML, Krance RA, Kun LE, Behm FG, et al: Low leukocyte counts with blast cells in cerebrospinal fluid of children with newly diagnosed acute lymphoblastic leukemia. N Engl J Med 329:314-319, 1993

29. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, et al: Proposals for the immunological classification of acute leukaemias. European group for the immunological characterisation of leukaemias (EGIL). Leukemia 9:1783-1786, 1995

30. Hiddemann W, Wormann B, Ritter J, Thiel E, Gohde W, Lahme B, et al: Frequency and clinical significance of DNA aneuploidy in acute leukemia. Ann N Y Acad Sci 468:227-240, 1986

31. Fletcher JA, Lynch EA, Kimball VM, Donnelly M, Tantravahi R, Sallan SE. Translocation t (9;22) is associated with extremely poor prognosis in intensively treated children with acute lymphoblastic leukemia. Blood 77:435-439, 1991

32. Rubnitz JE, Link MP, Shuster JJ, Carroll AJ, Hakami N, Frankel LS, et al: Frequency and prognostic significance of HRX rearrangements in infant acute lymphoblastic leukemia: a Pediatric Oncology Group study. Blood 84:570-573, 1994

33. Behm FG, Raimondi SC, Frestedt JL, Liu Q, Crist WM, Downing JR, et al: Rearrangement of the MLL gene confers a poor prognosis in childhood acute lymphoblastic leukemia, regardless of presenting age. Blood 87:2870-2877, 1996

34. Schlieben S, Borkhardt A, Reinisch I, Ritterbach J, Janssen JW, Ratei R, et al: Incidence and clinical outcome of children with BCR/ABL-positive acute lymphoblastic leukemia (ALL) . A prospective RT-PCR study based on 673 patients enrolled in the German pediatric multicenter therapy trials ALL-BFM 90 and CoALL-05-92. Leukemia 10:957-963, 1996

35. Borkhardt A, Cazzaniga G, Viehmann S, Valsecchi MG, Ludwig WD, Burci L, Mangioni S, et al: Incidence and clinical relevance of TEL/AML1 fusion genes in children with acute lymphoblastic leukemia enrolled in the German and Italian multicenter therapy trials. Associazione Italiana Ematologia Oncologia Pediatrica and the Berlin-Frankfurt-Munster Study Group. Blood 90:571-577, 1997

36. Trka J, Zuna J, Hrusak O, Kalinova M, Muzikova K, Lauschman H, et al: Impact of TEL/AML1-positive patients on age distribution of childhood acute lymphoblastic leukemia in Czech Republic. Leukemia 12:996-1007, 1998

37. Loh ML, Silverman LB, Young ML, Neuberg D, Golub TR, Sallan SE, et al: Incidence of TEL/AML1 fusion in children with relapsed acute lymphoblastic leukemia. Blood 15:4792-4797, 1998

38. Cave H, van der Werff ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J, et al: European Organization for Research and Treatment of Cancer–Childhood Leukemia Cooperative Group. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. N Engl J Med 339:591-598, 1998

39. van Dongen JJ, Seriu T, Panzer-Grümayer ER, Biondi A, Pongers-Willemse MJ, Corral L, et al: Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 352:1731-1738, 1998

40. Foroni L, Harrison JC, Hoffbrand AV, Potter MN: Investigation of minimal residual disease in childhood and adult acute lymphoblastic leukemia by molecular analysis. Br J Haematol 105:7-24, 1999

41. Ciudad J, San Miguel JF, López-Berges MC, Vidriales B, Valverde B, Ocqueteau M, et al: Prognostic value of immunophenotypic detection of minimal residual disease in acute lymphoblastic leukemia. J Clin Oncol 16:3774-3781, 1998

42. Coustan-Smith E, Sancho J, Hancock ML, Boyett JM,

Behm FG, Raimondi SC, et al: Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood 96:2691-2696, 2000

43. Dworzak MN, Froschl G, Printz D, Mann G, Pötschger U, Mühlegger N, et al: Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. Blood 99:1952-1958, 2002

44. Nyvold C, Madsen HO, Ryder LP, Seyfarth J, Svejgaard A, Clausen N, et al: Precise quantification of minimal residual disease at day 29 allows identification of children with acute lymphoblastic leukemia and an excellent outcome. Blood 99:1253-1258, 2002

45. Brisco MJ, Condon J, Hughes E, Neoh SH, Sykes PJ, Seshadri R, et al: Outcome prediction in childhood acute lymphoblastic leukaemia by molecular quantification of residual disease at the end of induction. Lancet 343:196-200, 1994

46. Borowitz MJ, Devidas M, Hunger SP, Bowman WP, Carroll AJ, Carroll WL, et al: Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood 111:5477-5485, 2008

47. Basso G, Veltroni M, Valsecchi MG, Dworzak MN, Ratei R, Silvestri D, et al. Risk of relapse of childhood acute lymphoblastic leukemia is predicted by flow cytometric measurement of residual disease on day 15 bone marrow. J Clin Oncol 27:5168-74, 2009

48. Flohr T, Schrauder A, Cazzaniga G, Panzer-Grümayer R, van der Velden V, Fischer S, et al: Minimal residual disease-directed risk stratification using real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements in the international multicenter trial AIEOP-BFM ALL 2000 for childhood acute lymphoblastic leukemia. Leukemia 22:771-782, 2008

49. Conter V, Bartram CR, Valsecchi MG, Schrauder A, Panzer-Grumayer R, Moricke A, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. Blood 115:3206-14, 2010

50. Bruggemann M, Schrauder A, Raff T, Pfeifer H, Dworzak M, Ottmann OG, et al. Standardized MRD quantification in European ALL trials: proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18-20 September 2008. Leukemia 24:521-35, 2010

51. Aricò M, Valsecchi MG, Rizzari C, Barisone E, Biondi A, Casale F, et al: Long-term results of the AIEOP-ALL-95 Trial for Childhood Acute Lymphoblastic Leukemia: insight on the prognostic value of DNA index in the framework of Berlin-Frankfurt-Münster based chemotherapy. J Clin Oncol 26:283-289, 2008

52. Trueworthy R, Shuster J, Look T, Crist W, Borowitz M, Carroll A, et al: Ploidy of lymphoblasts is the strongest predictor of treatment outcome in B-progenitor cell acute lymphoblastic leukemia of childhood: a Pediatric Oncology Group Study. J Clin Oncol 10:606-613, 1992

53. Heerema NA, Nachman JB, Sather HN, Sensel MG, Lee MK, Hutchinson R, et al: Hypodiploidy with less than 45 chromosomes confers adverse risk in childhood acute lymphoblastic leukemia: a report from the Childrens' Cancer Group. Blood 94:4036-4045, 1999

54. Harris MB, Shuster JJ, Carroll A, Look AT, Borowitz MJ, Crist WM, et al: Trisomy of leukemic cells chromosomes 4 and 10 identifies children with B-progenitor cell acute lymphoblastic leukemia with a very low risk of treatment failure: a Pediatric Oncology Group study. Blood 79:3316-3324, 1992

55. Nachman JB, Heerema NA, Sather H, Camitta B, Forestier E, Harrison CJ, et al: Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. Blood 110:1112-1125, 2007

56. Harbott J, Ritterbach J, Ludwig W-D, Bartram CR, Reiter A, Lampert F: Clinical significance of cytogenetic studies in childhood acute lymphoblastic leukemia: experience of the BFM trials. Recent Results in Cancer Research 131:123-132, 1993

57. Moorman AV, Ensor HM, Richards SM, Chilton L, Schwab C, Kinsey SE, et al. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. Lancet Oncol. 11:429-38, 2010

58. Pui CH, Rivera GK, Hancock ML, Raimondi SC, Sandlund JT, Mahmoud HH, et al: Clinical significance of CD10 expression in childhood acute lymphoblastic leukemia. Leukemia 7:35-40, 1993

59. Ludwig W-D, Harbott J, Bartram CR, Riehm H: Incidence and prognostic significance of immunophenotypic subgroups in childhood acute lymphoblastic leukemia: experience of the BFM study 86. In: Recent advances in cell biology of acute leukemia: impact on clinical diagnosis and therapy (Ludwig W-D, Thiel E, eds.), Berlin: Springer Verlag, 269-282, 1993

60. Pui CH, Boyett JM, Relling MV, Harrison PL, Rivera GK, Behm FG, et al: Sex differences in prognosis for children with acute lymphoblastic leukemia. J Clin Oncol 17:818-824, 1999

61. Gajjar A, Harrison PL, Sandlund JT, Rivera GK, Ribeiro RC, Rubnitz JE, et al: Traumatic lumbar puncture at diagnosis adversely affects outcome in childhood acute lymphoblastic leukemia. Blood 96:3381-3384, 2000

62. Riehm H, Reiter A, Schrappe M, Berthold F, Dopfer R, Gerein V, et al: The in vivo response on corticosteroid therapy as an additional prognostic factor in childhood acute lymphoblastic leukemia (therapy study ALL-BFM 83). Klin Pädiatr 199:151-160, 1987

63. Gaynon PS, Bleyer WA, Steinherz PG, Finklestein JZ, Littman P, Miller DR, et al: Day 7 marrow response and outcome for children with acute lymphoblastic leukemia and unfavorable presenting features. Med Pediatr Oncol 18:273-279, 1990

64. Janka-Schaub GE, Stührk H, Kortüm B, Graubner U, Jürgens H, Spaar HJ, et al: Bone marrow blast count at day 28 as the single most important prognostic factor in childhood acute lymphoblastic leukemia. Haematol Blood Transfus 34:233-237, 1992

65. Arico M, Basso G, Mandelli F, Rizzari C, Colella R, Barisone E, et al: Good steroid response in vivo predicts a favorable outcome in children with T-cell acute lymphoblastic leukemia. Cancer 75:1684-1693, 1995

66. Gajjar A, Ribeiro R, Hancock ML, Rivera GK, Mahmoud H, Sandlund JT, et al: Persistence of circulating blasts after 1 week of multiagent chemotherapy confers a poor prognosis in childhood acute lymphoblastic leukemia. Blood 86:1292-1295, 1995

67. Steinherz PG, Gaynon PS, Breneman JC, Cherlow JM, Grossman NJ, Kersey JH, et al: Cytoreduction and prognosis in acute lymphoblastic leukemia - the importance of early marrow response: report from the Childrens Cancer Group. J Clin Oncol 14:389-398, 1996

68. Manabe A, Ohara A, Hasegawa D, Koh K, Saito T, Kiyokawa N, et al: Significance of the complete clearance of peripheral blasts after 7 days of prednisolone treatment in children with acute lymphoblastic leukemia: the Tokyo Children's Cancer Study Group Study L99-15. Haematologica 93:1155-1160, 2008

69. Schrappe M, Reiter A, Ludwig WD, Harbott J, Zimmermann M, Hiddemann W, et al. Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. German-Austrian-Swiss ALL-BFM Study Group. Blood 95:3310-22, 2000

70. Moghrabi A, Levy DE, Asselin B, Barr R, Clavell L, Hurwitz C, et al. Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia. Blood 109:896-904, 2007

71. Tubergen DG, Gilchrist GS, O'Brien RT, Coccia PF, Sather HN, Waskerwitz MJ, et al: Improved outcome with delayed intensification for children with acute lymphoblastic leukemia and intermediate presenting features: a Childrens Cancer Group phase III trial. J Clin Oncol 11:527-537, 1993

72. van Dalen EC, Raphaël MF, Caron HN, Kremer LC. Treatment including anthracyclines versus treatment not including anthracyclines for childhood cancer. Cochrane Database Syst Rev. 2009 Jan 21; (1) :CD006647.

73. Childhood Acute Lymphoblastic Leukaemia Collaborative Group (CALLCG) . Beneficial and harmful effects of anthracyclines in the treatment of childhood acute lymphoblastic leukaemia: a systematic review and meta-analysis. Br J Haematol. 145:376-88, 2009

74. Duval M, Suciu S, Ferster A, Rialland X, Nelken B, Lutz P, et al. Comparison of Escherichia coli-asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer-Children's Leukemia Group phase 3 trial. Blood 99:2734-9, 2002

75. Kaspers GJ, Veerman AJ, Popp-Snijders C, Lomecky M, Van Zantwijk CH, Swinkels LM, et al: Comparison of the antileukemic activity in vitro of dexamethasone and prednisolone in childhood acute lymphoblastic leukemia. Med Pediatr Oncol 27:114-121, 1996

76. Jones B, Freeman AI, Shuster JJ, Jacquillat C, Weil M, Pochedly C, et al: Lower incidence of meningeal leukemia when prednisone is replaced by dexamethasone in the treatment of acute lymphocytic leukemia. Med Pediatr Oncol 19:269-275, 1991

77. Veerman AJ, Hählen K, Kamps WA, Van Leeuwen EF, De Vaan GA, Solbu G, et al: High cure rate with a moderately intensive treatment regimen in non-high-risk childhood acute lymphoblastic leukemia. Results of protocol ALL VI from the Dutch Childhood Leukemia Study Group. J Clin Oncol 14:911-918, 1996

78. Silverman LB, Gelber RD, Dalton VK, Asselin BL, Barr RD, Clavell LA, et al: Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood 97:1211-1218, 2001

79. Bostrom BC, Sensel MR, Sather HN, Gaynon PS, La MK, Johnston K, et al: Dexamethasone versus prednisone and daily oral versus weekly intravenous mercaptopurine for patients with standard-risk acute lymphoblastic leukemia: a report from the Children's Cancer Group. Blood 101:3809-3817, 2003

80. Igarashi S, Manabe A, Ohara A, Kumagai M, Saito T, Okimoto Y, et al: No advantage of dexamethasone over prednisolone for the outcome of standard- and intermediate-risk childhood acute lymphoblastic leukemia in the Tokyo Children's Cancer Study Group L95-14 protocol. J Clin Oncol 23:6489-6498, 2005

81. Mitchell CD, Richards SM, Kinsey SE, Lilleyman J, Vora A, Eden TO, et al: Benefit of dexamethasone compared with prednisolone for childhood acute lymphoblastic leukaemia: results of the UK Medical Research Council ALL 97 randomized trial. Br J Haematol 129:734-745, 2005

82. Hurwitz CA, Silverman LB, Schorin MA, Clavell LA, Dalton VK, Glick KM, et al: Substituting dexamethasone for prednisone complicates remission induction in children with acute lymphoblastic leukemia. Cancer 88:1964-1969, 2000

83. Waber DP, Carpentieri SC, Klar N, Silverman LB, Schwenn M, Hurwitz CA, et al: Cognitive sequelae in children treated for acute lymphoblastic leukemia with dexamethasone or prednisone. J Pediatr Hematol Oncol 22:206-213, 2000

84. Silverman LB, Gelber RD, Young ML, Dalton VK, Barr RD, Sallan SE. Induction failure in acute lymphoblastic leukemia of childhood. Cancer 85:1395-404, 1999

85. Oudot C, Auclerc MF, Levy V, Porcher R, Piguet C, Perel Y, et al. Prognostic factors for leukemic induction failure in children with acute lymphoblastic leukemia and outcome after salvage therapy: the FRALLE 93 study. J Clin Oncol 26:1496-503, 2008

86. Henze G, Fengler R, Reiter A, Ritter J, Riehm H. Impact of early intensive reinduction therapy on event-free survival in children with low-risk acute lymphoblastic leukemia. Hamatol Bluttransfus 33:483-8, 1990

87. Riehm H, Gadner H, Henze G, Kornhuber B, Lampert F, Niethammer D, et al. Results and significance of six randomized trials in four consecutive ALL-BFM studies. Hamatol Bluttransfus 33:439-50, 1990.

88. Reiter A, Schrappe M, Ludwig WD, Hiddemann W, Sauter S, Henze G, et al. Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. Blood 84:3122-33, 1994

89. Rizzari C, Valsecchi MG, Aricò M, Conter V, Testi A, Barisone E, et al: Associazione Italiano Ematologia Oncologia Pediatrica. Effect of protracted high-dose L-asparaginase given as a second exposure in a Berlin-Frankfurt-Münster-based treatment: results of the randomized 9102 intermediate-risk childhood acute lymphoblastic leukemia study–a report from the Associazione Italiana Ematologia Oncologia Pediatrica. J Clin Oncol 19:1297-1303, 2001

90. Pession A, Valsecchi MG, Masera G, Kamps WA, Magyarosy E, Rizzari C, et al: Long-term results of a randomized trial on extended use of high dose L-asparaginase for standard risk childhood acute lymphoblastic leukemia. J Clin Oncol 23:7161-7167, 2005

91. Nachman JB, Sather HN, Sensel MG, Trigg ME, Cherlow JM, Lukens JN, et al: Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. N Engl J Med 338:1663-1671, 1998

92. Seibel NL, Steinherz PG, Sather HN, Nachman JB, DeLaat C, Ettinger LJ, et al: Early postinduction intensification therapy improves survival for children and adolescents with high-risk acute lymphoblastic leukemia: a report from the Children's Oncology Group. Blood 111:2548-2555, 2008

93. Mattano LA, Sather HN, Trigg ME, Nachman JB: Osteonecrosis as a complication of treating acute lymphoblastic leukemia in children: a report from the Children's Cancer Group. J Clin Oncol 18:3262-3272, 2000

94. Kamps WA, Bökkerink JP, Hakvoort-Cammel FG, Veerman AJ, Weening RS, van Wering ER, et al: BFM-oriented treatment for children with acute lymphoblastic leukemia without cranial irradiation and treatment reduction for standard risk patients: results of DCLSG protocol ALL-8 (1991-1996). Leukemia 16:1099-1111, 2002

95. Conter V, Schrappe M, Arico M, Reiter A, Rizzari C, Dordelmann M, et al. Role of cranial radiotherapy for childhood T-cell acute lymphoblastic leukemia with high WBC count and good response to prednisone. Associazione Italiana Ematologia Oncologia Pediatrica and the Berlin- Frankfurt-Munster groups. J Clin Oncol 15:2786-91, 1997

96. Pui CH, Howard SC: Current management and challenges of malignant disease in the CNS in paediatric leukaemia. Lancet Oncol 9:257-268, 2008

97. Pui CH, Campana D, Pei D, Bowman WP, Sandlund JT, Kaste SC, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. The New England journal of medicine 360:2730-41, 2009

98. Sallan SE, Schrappe M, Silverman LB. Treating childhood leukemia without cranial irradiation. The New England journal of medicine 361:1310, 2009

99. Clarke M, Gaynon P, Hann I, Harrison G, Masera G, Peto R, et al: CNS-directed therapy for childhood acute lymphoblastic leukemia: Childhood ALL Collaborative Group overview of 43 randomized trials. J Clin Oncol 21:1798-1809, 2003

100. Mastrangelo R, Poplack D, Bleyer A, Riccardi R, Sather HN, D'Angio G: Report and recommendations of the Rome workshop concerning poor-prognosis acute lymphoblastic leukemia in children: Biologic bases for staging, stratification, and treatment. Med Pediatr Oncol 14:191-194, 1986

101. Bürger B, Zimmermann M, Mann G, Kühl J, Löning L, Riehm H, et al: Diagnostic cerebrospinal fluid (CSF) examination in children with acute lymphoblastic leukemia (ALL) : Significance of low leukocyte counts with blasts or traumatic lumbar puncture. J Clin Oncol 21:184-188, 2003

102. Cario G, Izraeli S, Teichert A, Rhein P, Skokowa J, Möricke A, et al: High interleukin-15 expression characterizes childhood acute lymphoblastic leukemia with involvement of the CNS. J Clin Oncol 25:4813-4820, 2007

103. Pinkel D. 'Allogeneic marrow transplantation in children with acute leukemia: a practice whose time has gone': twenty years later. Leukemia 23):2189-96, 2009

104. Schrauder A, von Stackelberg A, Schrappe M, Cornish J, Peters C. Allogeneic hematopoietic SCT in children with ALL: current concepts of ongoing prospective SCT trials. Bone Marrow Transplant. 2008 Jun;41 Suppl 2:S71-4.

105. Schrauder A, Reiter A, Gadner H, Niethammer D, Klingebiel T, Kremens B, et al. Superiority of allogeneic hematopoietic stem-cell transplantation compared with chemotherapy alone in high-risk childhood T-cell acute lymphoblastic leukemia: results from ALL-BFM 90 and 95. J Clin Oncol. 24:5742-9, 2006

106. Balduzzi A, Valsecchi MG, Uderzo C, De Lorenzo P, Klingebiel T, Peters C, et al: Chemotherapy versus allogeneic transplantation for very-high-risk childhood acute lymphoblastic leukaemia in first complete remission: comparison by genetic randomisation in an international prospective study. Lancet 366:635-642, 2005

107. Aricò M, Valsecchi MG, Camitta B, Schrappe M, Chessells J, Baruchel A, et al: Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med 342:998-1006, 2000

108. Giverhaug T, Loennechen T, Aarbakke J: Increased concentrations of methylated 6-mercaptopurine metabolites and 6-thioguanine nucleotides in human leukemic cells in vitro by methotrexate. Biochem Pharmacol 55:1641-1646, 1998

109. Dervieux T, Hancock ML, Evans WE, Pui CH, Relling MV: Effect of methotrexate polyglutamates on thioguanine nucleotide concentrations during continuation therapy of acute lymphoblastic leukemia with mercaptopurine. Leukemia 16:209-212, 2002

110. Rivard GE, Infante-Rivard C, Dresse MF, Leclerc JM,

Champagne J: Circadian time-dependent response of childhood lymphoblastic leukemia to chemotherapy: a longterm follow-up study of survival. Chronobiol Int 10:201-204, 1993

111. Schmiegelow K, Glomstein A, Kristinsson J, Salmi T, Schroder H, Bjork O: Impact of morning versus evening schedule for oral methotrexate and 6-mercaptopurine on relapse risk for children with acute lymphoblastic leukemia. Nordic Society for Pediatric Hematology and Oncology (NOPHO). J Pediatr Hematol Oncol 19:102-109, 1997

112. Sofianou-Katsoulis A, Khakoo G, Kaczmarski R: Reduction in bioavailability of 6-mercaptopurine on simultaneous administration with cow's milk. Pediatr Hematol Oncol 23:485-487, 2006

113. Relling MV, Hancock ML, Boyett JM, Pui CH, Evans WE: Prognostic importance of 6-mercaptopurine dose intensity in acute lymphoblastic leukemia. Blood 93:2817-2823, 1999

114. Conter V, Valsecchi MG, Silvestri D, Campbell M, Dibar E, Magyarosy E, et al: Pulses of vincristine and dexamethasone in addition to intensive chemotherapy for children with intermediate-risk acute lymphoblastic leukaemia: a multicentre randomised trial. Lancet 369:123-131, 2007

115. Childhood ALL Collaborative Group: Duration and intensity of maintenance chemotherapy in acute lymphoblastic leukaemia: overview of 42 trials involving 12000 randomised children. Lancet 347:1783-1788, 1996

116. Harms DO, Göbel U, Spaar HJ, Graubner UB, Jorch N, Gutjahr P, et al: Thioguanine offers no advantage over mercaptopurine in maintenance treatment of childhood ALL: results of the randomized trial COALL-92. Blood 102:2736-2740, 2003

117. Vora A, Mitchell CD, Lennard L, Eden TO, Kinsey SB, Lilleyman J, et al: Toxicity and efficacy of 6-thioguanine versus 6-mercaptopurine in childhood lymphoblastic leukaemia: a randomised trial. Lancet 368:1339-1348, 2006

118. Stork LC, Matloub Y, Broxson E, La M, Yanofsky R, Sather H, et al. Oral 6-mercaptopurine versus oral 6-thioguanine and veno-occlusive disease in children with standard-risk acute lymphoblastic leukemia: report of the Children's Oncology Group CCG-1952 clinical trial. Blood 115:2740-8, 2010

119. Henze G, Fengler R, Hartmann R. Chemotherapy for relapsed childhood acute lymphoblastic leukemia: results of the BFM Study Group. Haematol Blood Transfus 36:374-379, 1994

120. Tallen G, Ratei R, Mann G, Kaspers G, Niggli F, Karachunsky A, et al. Long-term outcome in children with relapsed acute lymphoblastic leukemia after time-point and site-of-relapse stratification and intensified short-course multidrug chemotherapy: results of trial ALL-REZ BFM 90. J Clin Oncol 28:2339-47, 2010

121. Gaynon PS, Qu RP, Chappell RJ, Willoughby ML, Tubergen DG, Steinherz PG, Trigg ME. Survival after relapse in childhood acute lymphoblastic leukemia: impact of site and time to first relapse: the Children's Cancer Group Experience. Cancer 82:1387-1395, 1998

122. Wheeler K, Richards S, Bailey C, Gibson B, Hann IM, Hill FG, et al: Comparison of bone marrow transplant and chemotherapy for relapsed childhood acute lymphoblastic leukaemia: The MRC UKALL X experience: Medical Research Council Working Party on Childhood Leukaemia. Br J Haematol 101:94-103, 1998

123. Borgmann A, von Stackelberg A, Hartmann R, Ebell W, Klingebiel T, Peters C, et al: Unrelated donor stem cell transplantation compared with chemotherapy for children with acute lymphoblastic leukemia in a second remission: a matched-pair analysis. Blood 101:3835-3839, 2003

124. Eapen M, Raetz E, Zhang M, Muehlenbein C, Devidas M, Abshire T, et al: Outcomes after HLA-matched sibling transplantation or chemotherapy in children with B-precursor acute lymphoblastic leukemia in a second remission: a collaborative study of the Children's Oncology Group and the Center for International Blood and Marrow Transplant Research. Blood 107: 4961-4967, 2006

125. Eckert C, Biondi A, Seeger K, Cazzaniga G, Hartmann R, Beyermann B, et al: Prognostic value of minimal residual disease in relapsed childhood acute lymphoblastic leukaemia. Lancet 358:1239-1241, 2001

126. Löning L, Zimmermann M, Reiter A, Kaatsch P, Henze G, Riehm H, et al. Secondary neoplasms subsequent to Berlin-Frankfurt-Münster therapy of childhood acute lymphoblastic leukemia: significantly lower risk without cranial radiotherapy. Blood. 2000 2000;95 (9) :2770-5

127. Mody R, Li S, Dover DC, Sallan S, Leisenring W, Oeffinger KC, Yasui Y, et al: Twenty-five-year follow-up among survivors of childhood acute lymphoblastic leukemia: a report from the Childhood Cancer Survivor Study. Blood 111:5515-5523, 2008

128. Landier W, Bhatia S, Eshelman DA, Forte KJ, Sweeney T, Hester AL, et al: Development of risk-based guidelines for pediatric cancer survivors: the Children's Oncology Group Long-Term Follow-Up Guidelines from the Children's Oncology Group Late Effects Committee and Nursing Discipline. J Clin Oncol 22:4979-4990, 2004

Update on Cytogenetics

Christine Harrison

In childhood acute lymphoblastic leukemia (ALL), the incidences of individual chromosomal abnormalities are well established. It is also known that their distribution varies according to age [1]. Especially in precursor-B ALL (BCP-ALL), they remain strong independent indicators of risk of relapse [2], while in T-ALL they contribute significantly to the understanding of the biology of the disease.

Structural chromosomal abnormalities in BCP-ALL

Among these abnormalities, those with the most significant impact for risk stratification for treatment are t(9;22)(q34;q11)/*BCR-ABL1* and rearrangements of the *MLL* gene. In particular this applies to t(4;11)(q21;q23)/*MLL-AFF1* (previously known as *MLL-AF4*). The prognosis of the other *MLL* partners may become significant in the future, particularly among infants [3]. The detection of these two abnormalities provides the basic criteria for the classification of high risk groups which is applicable to all treatment protocols. Other significant structural abnormalities include t(12;21)(p13;q22)/*ETV6-RUNX1* fusion, as well as t(1;19)(q23;p13.3)/*TCF3-PBX1* fusion. However, these are not used in risk stratification on all protocols. The *ETV6-RUNX1* fusion occurs in approximately 25% of younger children with BCP-ALL. These patients have an extremely good prognosis Among patients with *TCF3* rearrangements, those with *TCF3-PBX1* were originally regarded as poor risk on some treatment protocols, but on modern therapy they are classified as standard risk [4, 5]. In contrast the rare variant, t(17;19)(q22;p13)/*HLF-TCF3* fusion, has a dismal outcome on all therapies [6]. Thus its accurate identification is important.

Translocations involving *IGH@* at 14q32 are emerging as a significant subgroup in childhood ALL [7-10]. It is of interest that they occur more frequently in adolescents and, although numbers are small, they appear to have an inferior outcome. Currently they are studied for research purposes only, but their strong clinical associations may lead to the need for routine screening in the future.

The cytogenetic subgroup, intrachromosomal amplification of chromosome 21 (iAMP21), was identified during routine screening for the presence of the *ETV6-RUNX1* fusion by fluorescence *in situ* hybridization (FISH) [11, 12]. Patients are negative for the *ETV6-RUNX1* fusion, while in addition to the two normal copies of the *ETV6* signal, show multiple *RUNX1* signals (3 or more additional signals) with this probe. In metaphase, one signal is located to the normal chromosome 21, while the others are seen in tandem duplication along an abnormal chromosome 21 [13]. In interphase, the signals are clustered together, except for one signal representing the normal chromosome 21 which is usually located apart. Cytogenetics, multiple colour FISH and high resolution genomic arrays have shown that the morphology of the abnormal chromosome 21 is highly variable between patients and that the commonly amplified region always includes the *RUNX1* gene [13-15]. This abnormality was originally described as poor risk [12, 13, 16, 17], although the outcome has since been shown to be protocol dependent [18, 19]. Thus its accurate detection is important to guide therapy, at least in some protocols.

Numerical chromosomal abnormalities in BCP-ALL

Significant numerical abnormalities include: high hyperdiploidy (51-65 chromosomes) [20], near-haploidy (24-29 chromosomes) and low hypodiploidy (31-39 chromosomes) [21, 22]. High hyperdiploidy accounts for approximately 30% of childhood BCP-ALL and is characterised by the gain of specific chromosomes. It is

associated with a good prognosis in children. Near-haploidy and low hypodiploidy are rare, comprising <1% each of childhood ALL. Their characteristic features are the gain of specific chromosomes onto the haploid chromosome set and, in the majority of patients, the presence of a population of cells with an exact doubling of this chromosome number [21]. Both are linked to a poor outcome and are used to stratify patients as high risk.

Submicroscopic abnormalities in BCP-ALL

A significant discovery was the finding that the disruption of genes involved in B-cell development played an important role in leukaemogenesis in childhood BCP-ALL [23]. Approximately 40% of these patients had abnormalities of genes involved in the B-cell developmental pathway: *PAX5*, *TCF3*, *EBF1*, *LEF1*, *IKZF1* and *IKZF3*. Other genes frequently affected were those controlling cell cycle progression: *CDKN2A*, *CDKN1B* and *RB1* [24, 25]. Many of these deletions can be detected by FISH and/or genomic arrays. Whether there is a link between these genes and outcome has become a critical question [26]. In particular, the association of *IKZF1* deletions with a poor prognosis [27, 28] requires further validation in prospective independent and unselected trial based patient cohorts. Thus at present routine screening is not a recommendation.

Recently, a cryptic translocation, t(X;14)(p22;q32) or t(Y;14)(p11;q32), involving *IGH@* and *CRLF2* in the pseudoautosomal region (PAR1) of the sex chromosomes, and a deletion within PAR1, giving rise to the *P2RY8-CRLF2* fusion, have been reported [29-32]. They lead to overexpression of CRLF2, which has been defined as a novel, significant abnormality in BCP-ALL. *CRLF2* alterations, including activating mutations of the *CRLF2* receptor itself, are associated with activating *JAK* mutations resulting in constitutive activation of the JAK-STAT signaling pathway [29, 31-33]. Activation of this pathway has been associated with a worse prognosis in adults and children [34, 35] and has been highlighted as an important consideration for targeted therapy. Following further validation, the detection of *CRLF2* alterations may become a necessary diagnostic test.

Chromosomal and genetic changes in T-ALL

The chromosomal changes found in T-ALL are different from BCP-ALL. Visible cytogenetic changes are seen in approximately 50% of T-ALL patients. Cryptic translocations, for example t(5;14)(q35;q32) involving *TLX3*, and deletions, such as *TAL1*, may be detected by FISH using appropriate probes [36], considerably increasingly the abnormality detection rate. In T-ALL, translocations involving the T-cell receptor loci are found in approximately 35% of T-ALL by FISH [37]. They may result in oncogenes becoming juxtaposed to the promoter and enhancer elements of the *TCR* genes, leading to their aberrant expression and the development of T-ALL. Alternatively, aberrant expression of oncogenic transcription factors in T-ALL may result from loss of the upstream transcriptional mechanisms that normally down regulate the expression of these oncogenes during T-cell development [38, 39]. The formation of oncogenic fusion transcripts is rare in T-ALL. Translocations of this type include *MLL* fusions and *PICALM-MLLT10*, as well some rare rearrangements involving the tyrosine kinase gene, *ABL1*. Aberrant expression of one or more transcription factors is a critical component of the molecular pathogenesis of T-ALL. These include the class B basic helix-loop-helix (*bHLH*) genes *TAL1*, *TAL2*, *LYL1*, *OLIG2* and *MYC*, as well as genes involved in transcription regulation, for example, the cysteine-rich LIM-only domain, *LMO1* and *LMO2* genes. Abnormalities also affect the homeodomain genes, *TLX1* and *TLX3*, and members of the *HOXA* cluster. Mutations, particularly those involving *NOTCH1* and *FBXW7* are significant in T-ALL, together being found in approximately 70% of cases. Mutations and deletions of the X-linked tumor suppressor gene *PHF6* [40] and *PTPN2* [41] have recently been reported; the latter has been identified as a modulator of response to treatment. Chromosomal rearrangements and amplification of *MYB* at 6q23 has been found in approximately 8% T-ALL, which represents an interesting molecular target for therapy .

De Keersmaecker *et al* [44] classified the different T-ALL specific abnormalities into subgroups which defined four pathways based on different classes of mutations that: 1) provide a proliferative advantage; 2) impair differentiation

and survival; 3) affect the cell cycle; and 4) provide self renewal capacity. One interesting finding has been the strong interelationships between the different types of abnormalities in T-ALL. The T-ALL specific oncogenes may be upregulated by association with the promotor regions of either *TRA/D@* or *TRB@,* as well as other genes, for example *BCL11B* (and *CDK6*). In addition to these abnormalities *NOTCH1* mutations and deletions of *CDKN2A* may be present, indicating an interacting role for chromosomal abnormalities in T-ALL [45]. Interlaced with these four major classes of mutations is the molecular classification, which has emerged from gene expression profiling [46, 47]. It has identified several gene expression signatures indicative of arrest at specific stages of thymocyte development; a *LYL1* positive signature represents immature thymocytes (pro-T), *TLX1* positive represents early cortical thymocytes and *TAL1* correlates with late cortical thymocytes. Thus, gene expression profiling has improved our understanding of the biological heterogeneity of the disease, whilst revealing clinically relevant subtypes. In addition, molecular analysis has shown its capacity to elucidate significant pathways relevant to the future treatment of T-ALL. These findings have indicated that continued genetic analysis in T-ALL is important to further classify this heterogeneous disease.

References

1. Harrison CJ. Cytogenetics of paediatric and adolescent acute lymphoblastic leukaemia. Br J Haematol. 2009;144:147-156.
2. Moorman AV, Ensor HM, Richards SM, et al. Prognostic effect of chromosomal abnormalities in childhood B-cell precursor acute lymphoblastic leukaemia: results from the UK Medical Research Council ALL97/99 randomised trial. Lancet Oncol. 2010;11:429-438.
3. Pieters R, Schrappe M, De LP, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. Lancet. 2007;370:240-250.
4. Pui CH, Raimondi S, Hancock ML, et al. Immunologic, cytogenetic, and clinical characterization of childhood acute lymphoblastic leukemia with the t(1;19)(q23;p13) or its derivative. J Clin Oncol. 1994;12:2601-2606.
5. Kager L, Lion T, Attarbaschi A, et al. Incidence and outcome of TCF3-PBX1-positive acute lymphoblastic leukemia in Austrian children. Haematologica. 2007;92:1561-1564.
6. Hunger SP. Chromosomal translocations involving the E2A gene in acute lymphoblastic leukemia: clinical features and molecular pathogenesis. Blood. 1996;87:1211-1224.
7. Akasaka T, Balasas T, Russell LJ, et al. Five members of the CEBP transcription factor family are targeted by recurrent IGH translocations in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Blood. 2007;109:3451-3461.
8. Chapiro E, Russell L, Radford-Weiss I, et al. Overexpression of CEBPA resulting from the translocation t(14;19)(q32;q13) of human precursor B acute lymphoblastic leukemia. Blood. 2006;10 3560-3563.
9. Russell LJ, Akasaka T, Majid A, et al. t(6;14)(p22;q32): a new recurrent IGH@ translocation involving ID4 in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Blood. 2008;111:387-391.
10. Russell LJ, De Castro DG, Griffiths M, et al. A novel translocation, t(14;19)(q32;p13), involving IGH@ and the cytokine receptor for erythropoietin. Leukemia. 2009;23:614-617.
11. Harrison CJ, Moorman AV, Barber KE, et al. Interphase molecular cytogenetic screening for chromosomal abnormalities of prognostic significance in childhood acute lymphoblastic leukaemia: a UK Cancer Cytogenetics Group Study. Br J Haematol. 2005; 129:520-530.
12. Soulier J, Trakhtenbrot L, Najfeld V, et al. Amplification of band q22 of chromosome 21, including AML1, in older children with acute lymphoblastic leukemia: an emerging molecular cytogenetic subgroup. Leukemia. 2003;17:1679-1682.
13. Harewood L, Robinson H, Harris R, et al. Amplification of AML1 on a duplicated chromosome 21 in acute lymphoblastic leukemia: a study of 20 cases. Leukemia. 2003;17:547-553.
14. Robinson HM, Harrison CJ, Moorman AV, Chudoba I, Strefford JC. Intrachromosomal amplification of chromosome 21 (iAMP21) may arise from a breakage-fusion-bridge cycle. Genes Chromosomes Cancer. 2007;46:318-326.
15. Strefford JC, Van Delft FW, Robinson HM, et al. Complex genomic alterations and gene expression in acute lymphoblastic leukemia with intrachromosomal amplification of chromosome 21. Proc Natl Acad Sci USA. 2006;103:8167-8172.
16. Robinson HM, Broadfield ZJ, Cheung KL, et al. Amplification of AML1 in acute lymphoblastic leukemia is associated with a poor outcome. Leukemia. 2003;17:2249-2250.
17. Moorman AV, Richards SM, Robinson HM, et al. Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). Blood. 2007;109:2327-2330.
18. Attarbaschi A, Mann G, Panzer-Grumayer R, et al. Minimal residual disease values discriminate between low and high relapse risk in children with B-cell precursor acute

lymphoblastic leukemia and an intrachromosomal amplification of chromosome 21: the Austrian and German acute lymphoblastic leukemia Berlin-Frankfurt-Munster (ALL-BFM) trials. J Clin Oncol. 2008;26:3046-3050.

19. Heerema NA, Carroll AJ, Borowitz MJ, et al. Amplification of AML1 Does Not Impact Early Outcome of Children with Acute Lymphoblastic Leukemia (ALL) Treated with Risk-Directed Chemotherapy: A Report From the Children's Oncology Group (COG). 2009;114:2598-
20. Moorman AV, Richards SM, Martineau M, et al. Outcome heterogeneity in childhood high-hyperdiploid acute lymphoblastic leukemia. Blood. 2003;102:2756-2762.
21. Harrison CJ, Moorman AV, Broadfield ZJ, et al. Three distinct subgroups of hypodiploidy in acute lymphoblastic leukaemia. Bri J Haematol. 2004;125:552-559.
22. Nachman JB, Heerema NA, Sather H, et al. Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. Blood. 2007;110:1112-1115.
23. Mullighan CG, Goorha S, Radtke I, et al. Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature. 2007;446:758-764.
24. Kuiper RP, Schoenmakers EF, van Reijmersdal SV, et al. High-resolution genomic profiling of childhood ALL reveals novel recurrent genetic lesions affecting pathways involved in lymphocyte differentiation and cell cycle progression. Leukemia. 2007;21:1258-1266.
25. Strefford JC, Worley H, Barber K, et al. Genome complexity in acute lymphoblastic leukemia is revealed by array-based comparative genomic hybridization. Oncogene. 2007;26:4306-4318.
26. Den Boer ML, van Slegtenhorst M, De Menezes RX, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol. 2009;10:125-134.
27. Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009;360:470-480.
28. Kuiper RP, Waanders E, van der Velden VH, et al. IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. Leukemia. 2010.
29. Russell LJ, Capasso M, Vater I, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood. 2009;114:2688-2698.
30. Mullighan CG, Collins-Underwood JR, Phillips LA, et al. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet. 2009;41:1243-1246.
31. Hertzberg L, Vendramini E, Ganmore I, et al. Down syndrome acute lymphoblastic leukemia: a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the iBFM Study Group. Blood. 2009;115:1006-1017.
32. Yoda A, Yoda Y, Chiaretti S, et al. Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. Proc Natl Acad Sci U S A. 2009;107:252-257.
33. Chapiro E, Russell L, Lainey E, et al. Activating mutation in the TSLPR gene in B-cell precursor lymphoblastic leukemia. Leukemia. 2009;24:642-645.
34. Harvey RC, Mullighan CG, Chen IM, et al. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. Blood. 2010.
35. Cario G, Zimmermann M, Romey R, et al. Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. Blood. 2010.
36. van der Burg M, Poulsen TS, Hunger SP, et al. Split-signal FISH for detection of chromosome aberrations in acute lymphoblastic leukemia. Leukemia. 2004; 18:895-908.
37. Bergeron J, Clappier E, Cauwelier B, et al. HOXA cluster deregulation in T-ALL associated with both a TCRD-HOXA and a CALM-AF10 chromosomal translocation. Leukemia. 2006;20:1184-1187.
38. Ferrando AA, Herblot S, Palomero T, et al. Biallelic transcriptional activation of oncogenic transcription factors in T-cell acute lymphoblastic leukemia. Blood. 2004;103:1909-1911.
39. van Vlierberghe P, van GM, Beverloo HB, et al. The cryptic chromosomal deletion del(11)(p12p13) as a new activation mechanism of LMO2 in pediatric T-cell acute lymphoblastic leukemia. Blood. 2006;108:3520-3529.
40. Van Vlierberghe P, Palomero T, Khiabanian H, et al. PHF6 mutations in T-cell acute lymphoblastic leukemia. Nat Genet. 2010;42:338-342.
41. Kleppe M, Lahortiga I, El Chaar T, et al. Deletion of the protein tyrosine phosphatase gene PTPN2 in T-cell acute lymphoblastic leukemia. Nat Genet. 2010;42:530-535.
42. Clappier E, Cuccuini W, Kalota A, et al. The C-MYB locus is involved in chromosomal translocation and genomic duplications in human T-cell acute leukemia (T-ALL), the translocation defining a new T-ALL subtype in very young children. Blood. 2007;110:1251-1261.
43. Lahortiga I, De Keersmaecker K, Van Vlierberghe P, et al. Duplication of the MYB oncogene in T cell acute lymphoblastic leukemia. Nat Genet. 2007;39:593-595.
44. De Keersmaecker K, Marynen P, Cools J. Genetic insights in the pathogenesis of T-cell acute lymphoblastic leukemia. Haematologica. 2005;90:1116-1127.
45. Harrison CJ. Genetics of T-cell acute lymphoblastic leukemia. http://onlinehaematologicaorg/supplements/Hematology_Education_2007pdf. 2007.
46. Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell. 2002;1:75-87.
47. Ferrando AA, Look AT. Gene expression profiling in T-cell acute lymphoblastic leukemia. Semin Hematol. 2003;40:274-280.

Detection of Minimal Residual Disease in Acute Lymphoblastic Leukemia

Jacques J.M. van Dongen and Vincent H.J. van der Velden

Abstract

MRD diagnostics during the first three months of treatment has proven to be of high value in pediatric acute lymphoblastic leukemia (ALL), because of its potential to recognize subgroups that differ substantially in outcome. Consequently, MRD diagnostics is now being used for treatment intervention, both treatment intensification (including stem cell transplantation) and treatment reduction. However, the possibilities for recognition of MRD-based risk groups are dependent on the timing of the follow-up samples and the sensitivity of the applied MRD method. The currently available data indicate that two time points are needed for identification of both high-risk and low-risk patients and that a sensitivity and quantitative range of at least 10^{-4} are needed. Current 4-color flow cytometry can be used for identification of high-risk patients and the more sensitive PCR techniques can be used also for identification of true low-risk patients with very low relapse rates, who might profit from treatment reduction. The new development in standardized 8-color flow cytometry might well be able to compete with MRD-PCR methods.

1. Introduction

Until a decade ago, the prognostic classification of acute lymphoblastic leukemia (ALL) was mainly based on clinical, immunophenotypic, and molecular genetic characteristics at diagnosis, such as high white blood cell (WBC) counts, T-ALL phenotype, and the presence of a specific fusion gene aberration. For example, the *TEL-AML1* fusion gene is associated with good prognosis, whereas the *BCR-ABL* fusion gene and *MLL* gene aberrations are associated with poor prognosis. Nevertheless, the corresponding patient groups show limited differences in relapse-free survival. Apparently many other factors influence treatment outcome as well. For example, treatment compliance is still an underestimated factor and so far only limited insight has been obtained in polymorphisms in enzyme systems that influence pharmacodynamics. Also the effects of tissue distribution and consequences of reduced liver and kidney function can not yet fully be calculated. Finally, the relative contribution of each of these factors to the total therapy effectiveness is yet unknown.

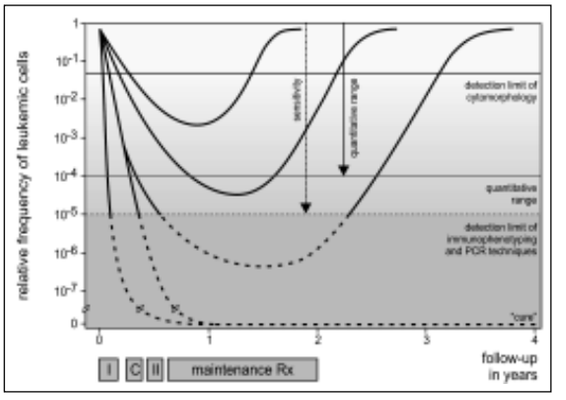

Figure 1.

Disappearance of leukemic cells from BM and the possible recurrence of the leukemia during or after treatment. The detection limits of the classical cytomorphological techniques as well as the flow cytometric and PCR techniques are indicated. The dotted red line represents the preferred sensitivity level (at least 10^{-4}, preferably 10^{-5}) and the solid red line represents the minimally required quantitative range, i.e. where reliable quantitative MRD results can be obtained.

However, the overall effect of all factors can be evaluated by measuring the kinetics of leukemic cell clearance from the bone marrow (BM) via application of sensitive techniques that allow detection of (very) low frequencies of leukemic cells, i.e. minimal residual disease (MRD). This allows the assessment of *in vivo* therapy effectiveness in individual patients (Figure 1). Over the last decade, large scale clinical studies in childhood and adult ALL have demonstrated that sensitive MRD diagnostics can recognize

MRD-based risk groups with unprecedentedly large differences in relapse-free survival. Several studies have shown that identification of truly low-risk patients requires MRD techniques that can detect one leukemic cell between at least 10^3 normal leukocytes ($\leq 10^{-3}$), preferably between 10^4 leukocytes ($\leq 10^{-4}$).[1, 2]

2. MRD methods

MRD methods should fulfill several requirements:

- sensitivity of at least 10^{-3}, preferably 10^{-4};
- applicability in majority of patients in the relevant treatment protocol;
- high reproducibility;
- possibility for interlaboratory standardization and international quality control.

At present, three methods fulfill most of the above requirements: flow cytometric immunophenotyping, polymerase chain reaction (PCR) -based detection of breakpoint fusion sites of chromosome aberrations at the RNA level (or DNA level), and PCR-based detection of junctional regions of rearranged immunoglobulin (Ig) and T-cell receptor (TCR) genes (Table 1).[1, 2]

Table 1. Characteristics of the three MRD techniques in ALL.

	Flow cytometric immunophenotyping[b]	PCR analysis	
		Ig-/TCR gene rearrangements	Fusion gene transcripts
Applicability[a]			
Precursor-B-ALL			
- infants	unknown[c]	70-80%	60-65% *(MLL)*[e]
- children	70-95%[b]	>95%	35-40%
- adults	70-85%	>90%[d]	40-45%[f]
T-ALL			
- children	>95%	>95%	10-20%
- adults	~95%	>90%[d]	10%
Sensitivity	$10^{-3} - 10^{-4}$	$10^{-4} - 10^{-5}$	$10^{-4} - 10^{-5}$
Advantages	- fast (1 to 2 days) - fairly patient-specific - also information about normal cells - technically relatively simple - "single cell"-analysis - viability of cells can be assessed	- high stability of DNA - highly patient-specific - DNA amount per cell is relatively stable	- stable target - (virtually) no background - relatively simple - relatively fast (2 to 3 days)
Disadvantages	- background of normal cells - immunophenotypic shifts - subclones - high sensitivity requires ~5 x 10^6 cells	- labor intensive and time consuming (slow) - background of normal cells - high level of complexity - loss of targets via continuous rearrangements (subclone formation)	- instability of RNA - variable expression levels (between patients and over time) - tumor specific, not patient specific (risk of contamination)

a. Percentage of patients for whom the MRD method can be applied.

b. Applicability increases when more than four fluorochromes are being used. Preferably standardized 8-color flow cytometry should be used.

c. Infant ALL is a rare disease for which no reliable flow cytometric MRD studies have been reported.

d. The number of rearranged Ig/TCR genes per patient is lower in adult ALL as compared to childhood ALL and the Ig/TCR gene rearrangements tend to be more immature in adult ALL.

e. Approximately 80% of infant ALL has an *MLL* gene rearrangement, for most of which (~70%) the breakpoint fusion site can be detected at the DNA level and might be used as sufficiently sensitive MRD-PCR target (60-65%)

sequence of the junctional regions can be determined. This sequence information allows the design of junctional region-specific oligonucleotides, either allele specific oligonucleotide (ASO) probes or ASO primers to be used for real-time quantitative PCR (RQ-PCR) analysis. Appropriate ASO primers or probes with sufficient sensitivity ($\leq 10^{-4}$) and sufficient quantitative range (down to 10^{-4}) can generally be developed in 60-70% of the rearrangements. This sensitivity is at least in part dependent on the size and composition of the junctional region, the rearranged gene segments, and the presence of comparable rearrangements in normal cells (Figure 2).

MRD-PCR analysis of Ig/TCR gene rearrangements is complex and time-consuming and requires extensive experience and knowledge about Ig/TCR gene rearrangement patterns. However, the advantages of high sensitivity, broad applicability, and high level of interlaboratory standardization have been the main reasons to use this MRD method in the vast majority of ALL protocols in Europe.[15, 26]

3. Clinical value of MRD detection in childhood ALL

The most significant application of MRD monitoring in childhood ALL is the evaluation of the initial response to chemotherapy, since numerous studies have demonstrated that low levels or absence of MRD after completion of induction therapy predicts excellent outcome (Figure 3).[16-21] The level of MRD-PCR positivity after induction therapy is independent of other clinically relevant risk factors (e.g., age, blast count at diagnosis, immunophenotype at diagnosis, presence of chromosome aberrations, response to prednisone, and classical clinical risk group assignment) and is the most powerful prognostic factor, so far.[16-21]

Depending on the treatment protocol, the sensitivity of the MRD technique, and the timing of the follow-up BM samples, MRD negativity is associated with overall relapse rates of only 2% to 10%.[16-21] On the other hand, several studies proved that high MRD levels at the end of induction treatment are associated with high relapse rates of 70% to 100%.[16-21]

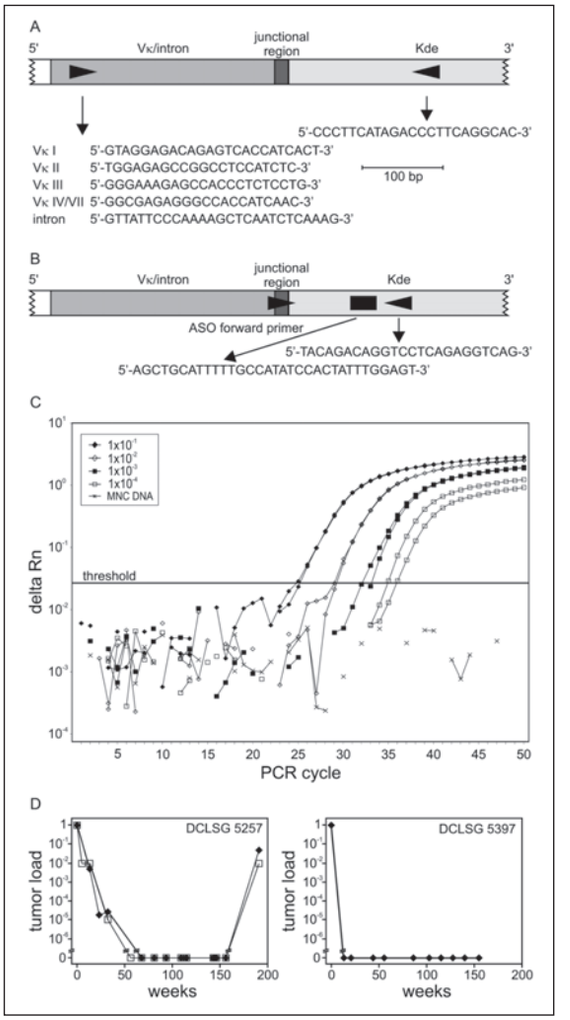

Figure 2.

RQ-PCR assay for detection of MRD using an IGK-Kde gene rearrangement as a patient-specific target. ***(A)*** *Schematic representation of an IGK-Kde rearrangement. The position and sequences of the primers used for target identification at diagnosis are indicated.* ***(B)*** *Sequences are given of the germline Taq-Man probe and the germline Kde reverse primer used for RQ-PCR analysis during follow-up of patients. All sequences are given from 5' to 3'. For each patient a patient specific forward primer is designed.* ***(C)*** *RQ-PCR analysis of the Vk-Kde rearrangement in a precursor-B-ALL patient. Ten-fold dilutions of the diagnostic sample in normal MNC DNA were analyzed at an annealing temperature of 60°C; a quantitative range of 10^{-4} was reached. Normal MNC DNA did not show amplification in any of the four wells tested.* ***(D)*** *Applicability of RQ-PCR analysis of IGK-Kde rearrangements for MRD detection in follow-up samples of two patients with precursor-B-ALL – an MRD high risk patient 5257 with high MRD levels ($\geq 10^{-3}$) at the early time points and a low risk patient 5397 with undetectable MRD already at the end of induction treatment. RQ-PCR analysis (black diamonds) was compared with classical dot blot analysis (open squares).*

2.1. Flow cytometric MRD detection

The application of flow cytometry for MRD detection is based on discrimination between ALL cells and normal leukocytes via leukemia associated phenotypic (LAP) characteristics, such as overexpression of CD10 and cross-lineage expression of myeloid antigens in precursor-B-ALL or co-expression of terminal deoxynucleotidyl transferase (TdT) and T-cell markers in T-ALL, a combination that normally is only found in the thymus.[1, 3]

Current 4-color flow cytometry reaches a fair sensitivity of 10^{-3} to 10^{-4} in most ALL patients. However, it should be noted that the detection of low frequencies of precursor-B-ALL cells in regenerating BM after induction therapy, after maintenance therapy, and after hematopoietic stem cell transplantation can be hampered by high frequencies of normal regenerating precursor-B-cells (up to 35%!). The extent and the pattern of B-cell regeneration in BM differs per treatment protocol, per phase of treatment, and seems to be dependent on the intensity of the preceding treatment: the more intensive the treatment, the more immature precursor-B-cells.[4, 5]

During the early phase of ALL treatment also immunophenotypic shifts may occur, which might be the direct result of the effect of the drugs on the expression level of various antigens with a shift to a more mature immunophenotype (e.g. CD10 decrease and CD20 increase) or may be related to drug-related cell kill.[6, 7] Logically, the background of regenerating precursor-B-cells and the drug-induced immunophenotypic shift reduce the sensitivity and specificity of the 4-color flow cytometric MRD methods. This particularly affects flow cytometric MRD detection during the second phase of induction therapy until the maintenance treatment, when the precursor-B-cell compartment is extended. For this reason, the 10^{-3} to 10^{-4} sensitivity levels can only be reached in the first 2 to 3 weeks of induction therapy. Furthermore, the interpretation of flowcytometric MRD data is still mainly experience-based and consequently very subjective. Finally, the detection of low MRD levels requires the detection of a cluster of at least 20 to 30 leukemic cells (for the precise quantitation even more cells are needed), implying that the immunostaining procedure has to start with approximately 5×10^6 cells, at least 5-fold more than currently used in highly sensitive MRD-PCR techniques.

2.2. Fusion gene aberration as MRD targets

Chromosome aberrations that result in fusion genes and fusion gene transcripts occur in 30 to 40% of pediatric precursor-B-ALL cases, particularly *TEL-AML1*, but also *BCR-ABL*, *MLL-AF4*, and *E2A-PBX1*.[8, 9] PCR analysis of fusion gene transcripts is fast and easy. However the limited applicability reduces its value for treatment protocols, unless a specific treatment arm or treatment protocol is applied for an ALL patient group that is defined according to the presence of a specific chromosome aberration, such as Imatimib treatment in *BCR-ABL* positive ALL patients.

Another example is the rare group of infant ALL cases, 80% of which have an *MLL* gene aberration.[10] Although more than 60 *MLL* partner genes have been identified so far[11], in most cases the *MLL* breakpoint fusion site can be detected at the DNA level, which can be used as patient-specific MRD-PCR target in 60 to 65% of infant ALL cases. Still, for the remaining 35 to 40% of cases another MRD method is needed.

2.3. MRD monitoring by PCR analysis of junctional regions

During early B- and T-cell differentiation the germline V, (D), and J gene segments of the Ig and TCR gene complexes rearrange, and each lymphocyte thereby obtains a specific combination of V- (D-) J segments that codes for the variable domains of Ig or TCR molecules. The random insertion and deletion of nucleotides at the junction sites of V, (D), and J gene segments make the junctional regions of Ig and TCR genes ''fingerprint-like'' sequences, which are different in each lymphocyte and thus also in each lymphoid malignancy, including ALL.[1, 2] Therefore, junctional regions can be used as tumor-specific targets for MRD-PCR analysis. If appropriate primer sets are applied, Ig/TCR gene rearrangements can be found in more than 95% of ALL patients.[12] Precursor-B-ALL might contain the following rearrangements: *IGH* (>95%, mainly VH-JH), *IGK* (~65%, mainly Kde), *IGL* (15-20%), *TCRB* (~35%), *TCRG* (~55%), *TCRD* (~40%), and Vδ2-Jα29 (40-45%).[13, 14] T-ALL might contain the following rearrangements: *TCRB* (~90%), *TCRG* (~95%), *TCRD* (~55%), and *IGH* (20-25%, mainly DH-JH).[13, 14]

For each of the indentified Ig/TCR gene rearrangements the precise nucleotide

MRD analysis at a single time point gives highly significant prognostic information, but a single time point is generally not sufficiently precise to define both MRD-based low-risk and high-risk groups. Depending on the MRD study, the end-of-induction MRD status either identifies only patients at low risk of relapse or more frequently identifies exclusively high-risk patients. In contrast, combined information on MRD levels at the end of induction treatment and before consolidation treatment is significantly superior to single time point measurement, which was first demonstrated by the International BFM Study Group (I-BFM-SG). This combined MRD information distinguished patients at low risk with MRD negativity at both time points (5-year relapse rate of 2%); from patients at high risk with an intermediate (10^{-3}) or high ($=10^{-2}$) degree of MRD at both time points (5-year relapse rate of 80%), and the remaining patients at intermediate risk (5-year relapse rate of 22%) (Figure 3).

MRD-based risk-group distribution in the I-BFM-SG study was even more striking in T-ALL: with fewer (~25%) low-risk patients with virtually no relapses, more (~25%) high-risk patients uniformly relapsing, and approximately 50% intermediate-risk patients with 25% relapses.[22]

The recently completed MRD study of the INTERFANT-99 protocol showed that also in this relatively homogeneous group of infant ALL cases (80% precursor-B-ALL with *MLL* gene aberrations), MRD-based subgroups can be identified that differ significantly in outcome (Figure 3B).[23]

In relapsed ALL patients, MRD diagnostics appears to be valuable as well, because rapid decrease of leukemic cells to low levels in the first five weeks of treatment is associated with good outcome.[24] Furthermore, in patients undergoing stem cell transplantation (SCT), MRD diagnostics can recognize three prognostic groups according to the pre-SCT MRD levels in bone marrow: no MRD detectable, low MRD levels, and high MRD levels with 5-year event-free survival (EFS) of 70 to 80%, 35 to 40% and 9 to 20%, respectively.[25]

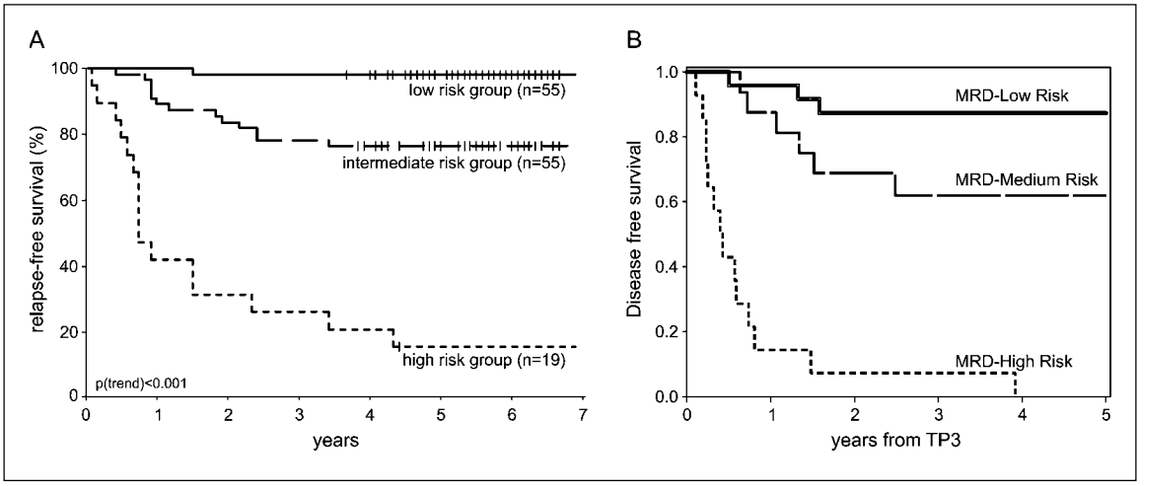

Figure 3.

Relapse-free survival in childhood ALL (DCOG-ALL8/BFM90 protocol) and infant ALL (INTERFANT99 protocol.[16,23] In the childhood ALL patients a large group of 43% low risk patients could be identified with only 3% of all relapses. Even in the infant ALL patients a group with a relatively low relapse rate of 13% could be identified.

3.1. MRD-based treatment intervention

Despite the overwhelming amount of data concerning the prognostic value of MRD diagnostics, major differences exist between clinical MRD studies, which exclude uniform guidelines for MRD-based treatment interventions.

Nevertheless, in several ongoing treatment protocols, the MRD-based high-risk group (~10% of childhood ALL) is subject to further treatment intensification, including SCT in first remission. Whereas the MRD-based high-risk

groups seem fairly comparable between the reported studies, the MRD-based low-risk group is much poorer defined. This group varies between studies from 35 to 40% of all patients (with 2 to 5% relapse rate) to 80% of all patients (with 10% relapse rate), and consequently harboring only 3% of all relapses versus more than 50% of all relapses, respectively.

The major differences between MRD-based low-risk patients can at least in part be explained by differences in the sensitivity and quantitative range of the applied MRD methods, but also differences in treatment protocol and timing of the analyzed follow-up samples play a role.

Logically, it is not possible to reduce treatment in a patient group that harbors many relapses.

However, in case of a very low relapse rate (≤3%), an MRD-based low-risk group might be treated with low-intensive treatment protocols, as is currently being done in the DCOG-ALL10 protocol.

4. Summary

Each of the three MRD methods has its specific advantages and disadvantages. The choice of the MRD method will be mainly dependent on its sensitivity and quantitative range, its applicability, and the possibilities of interlaboratory standardization.

For example, current 4-color flow cytometric MRD detection has a limited sensitivity of 10^{-3} to 10^{-4} and is not easy to standardize, but these disadvantages are less relevant for a single-center MRD study which aims at recognition of MRD-based high-risk patients only. However, an international multi-center study that aims at treatment reduction in MRD-based low-risk patients will most likely use PCR analysis of Ig/TCR gene rearrangements.

For reasons of standardization of the Ig/TCR-based MRD-PCR diagnostics, the EuroMRD (former ESG-MRD-ALL) Consortium was initiated in 2001. This Consortium now includes 42 MRD laboratories and each year organizes two quality control (QC) rounds, followed by an interactive meeting to discuss the QC results as well as the standardized MRD methods and standardized interpretation and reporting of MRD results.

The same high level of standardization has not yet been achieved for flow cytometric MRD detection. Therefore the EuroFlow Consortium has now initiated a comparable process for the design, standardization, and clinical evaluation of 8-color MRD tubes for the various immunophenotypic ALL subgroups, as defined by the 8-color EuroFlow diagnosis & classification antibody panels. It is anticipated that the combined usage of standardized 8-color MRD tubes and the novel Infinicyt software will allow sensitive and objective (partly automated) flowcytometric MRD diagnostics for virtually all ALL patients. If indeed successful, this approach will combine high sensitivity, broad applicability, high speed (results within 1 to 2 days), and full interlaboratory standardization.

References

1. Szczepanski T, Orfão A, Van der Velden VHJ, San Miguel JF, Van Dongen JJM. Minimal residual disease in leukaemia patients. Lancet Oncol 2001; 2: 409-417.
2. Van Dongen JJM, Szczepanski T, Van der Velden VHJ. Minimal residual disease. In: Kaspers GJL, Coiffier B, Heinrich MC, Estey E, eds. Innovative Leukemia and Lymphoma Therapy 2008. New York: Informa Healthcare; 2008: 45-84 (ISBN-10 0-8493-5083-2; ISNB-13 987-0-8493-5083-2).
3. Campana D, Coustan-Smith E. Advances in the immunological monitoring of childhood acute lymphoblastic leukaemia. Best Pract Res Clin Haematol 2002; 15: 1-19.
4. Van Lochem EG, Van der Velden VHJ, Wind H, te Marvelde JG, Westerdaal NAC, Van Dongen JJM. Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: reference patterns for age-related changes and disease-induced shifts. Clinical Cytometry 2004; 60B: 1-13.
5. Van Wering ER, Van der Linden-Schrever BE, Szczepanski T, Willemse MJ, Baars EA, Van Wijngaarde-Schmitz HM, Kamps WA, Van Dongen JJM. Regenerating normal B-cell precursors during and after treatment of acute lymphoblastic leukaemia: implications for monitoring of minimal residual disease. Br J Haematol 2000; 110: 139-146.
6. Gaipa G, Basso G, Maglia O, Leoni V, Faini A, Cazzaniga G, Bugarin C, Veltroni M, Michelotto B, Ratei R, Coliva T, Valsecchi MG, Biondi A, Dworzak MN. Drug-induced immunophenotypic modulation in childhood ALL: implications for minimal residual disease detection. Leukemia 2005; 19: 49-56
7. Van der Sluijs-Gelling AJ, Van der Velden VHJ, Roeffen ET, Veerman AJ, Van Wering ER. Immunophenotypic modulation in childhood precursor-B-ALL can be mimicked in vitro and is related to the induction of cell death. Leukemia 2005; 19: 1845-1847.

8. Van Dongen JJM, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, Gottardi E, Rambaldi A, Dotti G, Griesinger F, Parreira A, Gameiro P, Diaz MG, Malec M, Langerak AW, San Miguel JF, Biondi A. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 1999;13:1901-1928.

9. Gabert J, Beillard E, Van der Velden VHJ, Bi W, Grimwade D, Pallisgaard N, Barbany G, Cazzaniga G, Cayuela JM, Cavé H, Pane F, Aerts JLE, De Micheli D, Thirion X, Pradel V, González M, Viehmann S, Malec M, Saglio G, Van Dongen JJM. Standardization and quality control studies of "real-time" quantitative reverse transcriptase polymerase chain reaction (RQ-PCR) of fusion gene transcripts for minimal residual disease detection in leukemia - A Europe Against Cancer Program. Leukemia 2003; 17: 2318-2357.

10. Jansen MW, Corral L, Van der Velden VHJ, Panzer-Grumayer R, Schrappe M, Schrauder A, Marschalek R, Meyer C, Den Boer ML, Hop WJ, Valsecchi MG, Basso G, Biondi A, Pieters R, Van Dongen JJM. Immunobiological diversity in infant acute lymphoblastic leukemia is related to the occurrence and type of MLL gene rearrangement. Leukemia 2007; 21: 633-641.

11. Meyer C, Kowarz E, Hofmann J, Renneville A, Zuna J, Trka J, Ben Abdelali R, Macintyre E, De Braekeleer E, De Braekeleer M, Delabesse E, de Oliveira MP, Cavé H, Clappier E, van Dongen JJM, Balgobind BV, van den Heuvel-Eibrink MM, BeverlooHB, Panzer-Grümayer R, Teigler-Schlegel A, Harbott J, Kjeldsen E, Schnittger S, Koehl U, Gruhn B, Heidenreich O, Chan LC, Yip SF, Krzywinski M, Eckert C, MörickeA, Schrappe M, Alonso CN, Schäfer BW, Krauter J, Lee DA, Zur Stadt U, Te Kronnie G, Sutton R, Izraeli S, Trakhtenbrot L, Lo Nigro L, Tsaur G, Fechina L, Szczepanski T, Strehl S, Ilencikova D, Molkentin M, Burmeister T, Dingermann T, Klingebiel T, Marschalek R. New insights to the MLL recombinome of acute leukemias. Leukemia 2009;23:1490-1499.

12. Van Dongen JJM, Langerak AW, Brüggemann M, Evans PA, Hummel M, Lavender L, Delabesse E, Davi F, Schuuring E, Garcia Sanz R, Van Krieken JHJM, Droese J, Gonzalez Diaz D, Bastard D, White H, Spaargaren M, Gonzáles M, Parreira A, Smith J, Morgan G, Kneba M, Macintyre EA. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations. Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 2003; 17: 2257-2317.

13. Van der Velden VHJ, Van Dongen JJM. MRD detection in acute lymphoblatic leukemia patients using Ig/TCR gene rearrangements as targets for real-time quantitative PCR. In: So CWE, ed. Leukemia: Methods in Molecular Biology. New York: Humana Press; 2009; 538: 115-150. (ISBN 978-1-58829-989-5 / e-ISBN 978-1-57945-418-6).

14. Van der Velden VHJ, Szczepanski T, Wijkhuijs JM, Hart PG, Hoogeveen PG, Hop WC, Van Wering ER, Van Dongen JJM. Age-related patterns of immunoglobulin and T-cell receptor gene rearrangements in precursor-B-ALL: implications for detection of minimal residual disease. Leukemia 2003; 17: 1834-1844.

15. Van der Velden VHJ, Cazzaniga G, Schrauder A, Hancock J, Bader P, Panzer-Grumayer ER, Flohr T, Sutton R, Cave H, Madsen HO, Cayuela JM, Trka J, Eckert C, Foroni L, Zur Stadt U, Beldjord K, Raff T, Van der Schoot CE, Van Dongen JJM. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. Leukemia 2007; 21:604-611.

16. Van Dongen JJM, Seriu T, Panzer-Grümayer ER, Biondi A, Pongers-Willemse MJ, Corral L, Stolz F, Schrappe M, Masera G, Kamps WA, Gadner H, Van Wering ER, Ludwig W-D, Basso G, De Bruijn MAC, Cazzaniga G, Hettinger K, Van der Does-van den Berg A, Hop WCJ, Riehm H, Bartram CR. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. Lancet 1998; 352:1731-1738.

17. Coustan-Smith E, Behm FG, Sanchez J, Boyett JM, Hancock ML, Raimondi SC, Rubnitz JE, Rivera GK, Sandlund JT, Pui CH, Campana D. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet 1998; 351:550-554.

18. Cave H, Van der Werff ten Bosch J, Suciu S, Guidal C, Waterkeyn C, Otten J, Bakkus M, Thielemans K, Grandchamp B, Vilmer E. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer—Childhood Leukemia Cooperative Group. N Engl J Med 1998; 339: 591-598.

19. Coustan-Smith E, Sancho J, Hancock ML, Boyett JM, Behm FG, Raimondi SC, Sandlund JT, Rivera GK, Rubnitz JE, Ribeiro RC, Pui CH, Campana D. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood 2000; 96:2691-2696.

20. Brüggemann M, Raff T, Flohr T, Gokbuget N, Nakao M, Droese J, Luschen S, Pott C, Ritgen M, Scheuring U, Horst HA, Thiel E, Hoelzer D, Bartram CR, Kneba M; German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. Blood 2006; 107:1116-1123.

21. Borowitz MJ, Devidas M, Hunger SP, Bowman WP, Carroll AJ, Carroll WL, Linda S, Martin PL, Pullen DJ, Viswanatha D, Willman CL, Winick N, Camitta BM; Children's Oncology Group. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. Blood 2008; 111: 5477-85.

22. Willemse MJ, Seriu T, Hettinger K, d'Aniello E, Hop WC, Panzer-Grumayer ER, Biondi A, Schrappe M, Kamps WA, Masera G, Gadner H, Riehm H, Bartram CR, Van Dongen JJM. Detection of minimal residual disease identifies differences in treatment response between T-ALL and precursor B-ALL. Blood 2002; 99: 4386-4393.

23. Van der Velden VHJ, Corral L, Valsecchi MG, Jansen MW, De Lorenzo P, Cazzaniga G, Panzer-Grümayer ER, Schrappe M, Schrauder A, Meyer C, Marschalek R, Nigro

LL, Metzler M, Basso G, Mann G, Den Boer ML, Biondi A, Pieters R, Van Dongen JJM; Interfant-99 Study Group. Prognostic significance of minimal residual disease in infants with acute lymphoblastic leukemia treated within the Interfant-99 protocol. Leukemia 2009;23:1073-1079.

24. Eckert C, Biondi A, Seeger K, Cazzaniga G, Hartmann R, Beyermann B, Pogodda M, Proba J, Henze G. Prognostic value of minimal residual disease in relapsed childhood acute lymphoblastic leukaemia. Lancet 2001; 358: 1239-1241.

25. Krejci O, Van der Velden VH, Bader P, Kreyenberg H, Goulden N, Hancock J, Schilham MW, Lankester A, Revesz T, Klingebiel T, Van Dongen JJM. Level of minimal residual disease prior to haematopoietic stem cell transplantation predicts prognosis in paediatric patients with acute lymphoblastic leukaemia: a report of the Pre-BMT MRD Study Group. Bone Marrow Transplant 2003; 32:849-851.

26. Van der Velden VHJ, Panzer-Grumayer ER, Cazzaniga G, Flohr T, Sutton R, Schrauder A, Basso G, Schrappe M, Wijkhuijs JM, Konrad M, Bartram CR, Masera G, Biondi A, Van Dongen JJM. Optimization of PCR-based minimal residual disease diagnostics for childhood acute lymphoblastic leukemia in a multi-center setting. Leukemia 2007; 21:706-713.

Infant Acute Lymphoblastic Leukemia

Rob Pieters

Introduction

Acute lymphoblastic leukemia (ALL) in infants under 12 months of age accounts for about 4% of childhood ALL and differs from ALL in older children with respect to immunophenotypic, cytogenetic and molecular genetic features. In contrast to the predominance of male sex in older children with ALL, there is a slight predominance of girls in infant ALL (1). In infant leukemia, all necessary genetic leukemogenic events may have occurred in utero, illustrated by the very early onset of infant ALL and the high rate of concordance of leukemia in monozygotic twins if one of the children developed leukemia during infancy. *MLL-AF4* fusion sequences have been detected in the Guthrie cards from children who were diagnosed with ALL in infancy (2).

Infants have a higher tumor load (median white blood cell count at diagnosis of 100 x 10^9/L) and more often central nervous system (CNS) involvement (15%) than older children with ALL (1).

Two-thirds of infant ALL has the immature CD10-negative B-lineage precursor ALL (proB ALL). Mature B-lineage ALL is an exceptional finding; T-lineage ALL is present in only 4% of cases (1). Infant ALL cells often express myeloid-associated antigens. Intraclonal switch from B-lineage to monocytic lineage leukemia has been described in infants.(3) These data illustrate that infant ALL arises from an immature precursor cell that is not fully committed to lymphoid differentiation.

Genetics

Trisomy 21 is a predisposing factor in the development of leukemia at young age, but if children with Down syndrome develop leukemia in the first year of life this is always myeloid leukaemia and never ALL. Cytogenetic abnormalities that occur relatively frequent in older children, such as hyperdiploidy and *TEL/AML1* fusion, but also the Philadelphia translocation t(9;22) and the t(1;19), are rare in infant ALL. About 80% of infant ALL cases carry translocations of the MLL gene. The t(4;11)(q21;q23) is found in 50% of the MLL gene rearranged cases, the t(11;19)(q23;p13) in 20% and the t(9;11)(p22;q23) in 10% (1), (4). The t(9;11) occurs in older infants than the t(4;11) and t(11;19) (4) and is associated with a more mature immunoglobulin rearrangement pattern (5). Many other partner chromosomes have been reported, occurring together in 10-20% of cases. The split-signal FISH method detects any type of MLL gene translocation and is therefore advised as a first screening technique (6).

The *MLL* gene encodes a member of the trithorax protein family, regulating transcription mediated by various functional domains. Disruption of the *MLL* gene leads to deregulated gene expression(7). MLL-rearranged ALL displays a unique expression profile that is clearly distinguishable from other ALL subtypes (8), (9) Moreover, in a recent study we demonstrated that, apart from a fundamental signature shared by all MLL-rearranged infant ALL samples, each type of MLL translocation is associated with a translocation-specific gene expression signature. We also showed the existence of 2 distinct subgroups among t(4;11)-positive infant ALL cases characterized by the absence or presence of HOXA expression, and that patients lacking HOXA expression are at extreme high risk of disease relapse (10). Highly characteristic for MLL fusion proteins is the loss of the H3K4 methyltransferase (SET) domain, which results in aberrant histone modifications, and hence altered chromatin remodeling. (11).(12) This results in abnormal promoter methylation patterns, and abnormal gene expression favoring malignant transformation. Recently performed genome-wide methylation studies in our laboratory showed that patients

carrying t(4;11) or t(11;19) exhibit extensive abnormal promoter methylation, directly influencing gene expression (50). In contrast, infant ALL patients carrying wild-type *MLL* genes or t(9;11) displayed DNA methylation patterns that closely resembled normal bone marrow. Moreover, apart from an overlapping promoter methylation profile shared by t(4;11) and t(11;19)-positive infant ALL patients, we also found distinct DNA methylation patterns that specifically associate with either translocation t(4;11) or t(11;19). This is in line with a recently postulated model proposing that different MLL fusion proteins lead to varying histone modifications directed by the *MLL* fusion partner. (11). These recent findings suggest that *MLL*-rearranged infant ALL can be considered an epigenetic malignancy, and that epigenetic therapies may be an attractive new therapeutic option (see below).

MicroRNAs (miRNAs) control the expression of protein-coding genes in normal hematopoietic cells. and, consequently, aberrant expression may contribute to leukemogenesis. To identify miRNAs relevant to MLL rearranged ALL, we recently cloned known and new miRNA genes expressed in patients' leukemia cells. Eight miRNAs were differentially expressed between MLL and non-MLL precursor B-ALL cases. The expression of miR-196b was 500-fold higher in MLL-rearranged ALL compared with the expression level in other precursor B-ALL cases whereas miR-708 was 500-fold lower expressed in MLL-rearranged ALL. The expression did not correlate with the maturation status of leukemia cells. (13). The miR-196b gene is located in the HOXA cluster at chromosome 7p15. It has been suggested that transcriptional activation of this cluster is caused by MLL binding and subsequent H3K4 methylation of associated histones. (14) In line with the fact that miR-196b is mapped between HOXA9 and HOXA10, we observed that miR-196b expression correlated with the expression of HOXA9 and HOXA10 in MLL-rearranged ALL. However, the high expression of miR-196b appeared not exclusively MLL-driven but was also found in other types of leukemia with aberrant activation of HOXA-genes. Since miR-196b has been shown to exert oncogenic activity in bone marrow progenitor cells, these observations imply a potential role for miR-196b in the underlying biology of all HOXA-activated leukemias (15).

Prognostic factors

The presence of MLL gene rearrangements, the absence of CD10 expression, and a high WBC are highly correlated with each other and are inversely related to the age of the infant. The poor prognosis of infant ALL has been associated with many factors in univariate analyses (1), (16) In the large Interfant study, multivariate analysis showed that the presence of MLL rearrangements and age < 6 months are the most important factor predicting a poor outcome followed by a WBC > 300 x 10e9/L and a poor prednisone response (4). Compared with the 9-12 month cohort, the hazard ratio for any event was 3.05, 2.25 and 1.64 for 0-3, 3-6 and 6-9 months cohorts, respectively. MLL rearranged cases showed a 3.1-fold increased risk of an event compared to MLL germline cases. This increased risk was the same regardless of the type of MLL rearrangement (4). These risk factors are now used for stratification in the Interfant-06 study.

Drug resistance

Leukemic cells from infants with MLL gene rearranged ALL cells grow better on stromal cell layers in vitro (17), have a higher leukemic cell recovery when inoculated into SCID mice (18) and are more resistant to cell death resulting from serum deprivation in vitro (19) compared with cells from other children with ALL. Infant ALL cells are more resistant in vitro to prednisolone and L-asparaginase than cells from older children with ALL (20) and infant ALL more frequently showes a poor response to prednisone than ALL in older children (21). The mechanisms of resistance are not known but recently we showed that overexpression of MCL-1 may contribute to this. Inhibition of MCL-1 by shRNA or by drugs sensitized MLL rearranged ALL cells from infants to glucocorticoids (22), (23). Infant ALL cells do not express higher levels of the multidrug resistance genes BCRP, MDR1, MRP1 and LRP/MVP than other ALL subtypes (24).

Although relatively resistant to several chemotherapeutic drugs, infant ALL cells are more sensitive to cytarabine (Ara-C) and 2-CdA (2-chlorodeoxyadenosine or cladribine)

compared with cells from older children with ALL (20), (25). Sensitivity to Ara-C in infant ALL appeared not to be directly associated with rearrangements of the *MLL* gene, as both *MLL* rearranged and *MLL* germ line infant ALL cases appeared equally sensitive to this drug *in vitro* (26). The Ara-C sensitivity is most likely due to the high expression of the human equilibrative nucleoside transporter 1 (hENT1)(27), on which Ara-C is mainly dependent to permeate the cell membrane. However, at high-dose Ara-C regimens, Ara-C also enters the cell by passive diffusion. Improved outcomes have been reported for infant ALL patients when high-dose Ara-C was implemented during the consolidation phase (28), (29). Also, improved outcome for adult pro-B ALL cases was observed with intensified post-remission therapy including high-dose Ara-C/mitoxantrone (30). Based upon these data, the collaborative Interfant-99 and Interfant-06 protocols added both low and high-dose Ara-C on top of a ALL based chemotherapy schedule (4).

Treatment

Treatment results. The results of studies on infant ALL published in the last decade show an EFS rate of ~50% or lower (table 1). Studies included low patient numbers with the exception of the Interfant-99 study that included 500 patients from 22 countries and achieved a long-term EFS of 47% and survival of 55% (4). The complete remission rate in infant ALL is 93-97% (table 1). Toxicity after remission induction is not the major problem: 4% of infants die from therapy toxicity while being in remission (1), (16). The major cause of treatment failure is relapse: about half of the patients experience a relapse, which involved the bone marrow in 80% of cases, the CNS in 30% and the testes in 8%. The majority of relapses occur very early during the first year of treatment already (4).

Comparison of treatment protocols.

Table 1. Outcome of infant ALL in Interfant-99 study and other published studies

Study group	CR rate	EFS/survival timepoint	EFS (SE)	Survival (SE)	Number patients	Reference
DFCI (1985-95)	96%	4 yr	54% (11%)	-	23	(28)
MLL-96 and MLL 98		5 yr	50%	61%	102	(39)
Interfant-99	94%	4 yr	47% (2.6%)	55.3% (2.7%)	482	(4)
AIEOP-91/95	96%	5 yr	45% (95% CI 31-58%)	-	52	(35)
BFM	95%	6 yr	43% (5%)	48% (6%)	105	(21)
EORTC-CLCG	86%	4 yr	43% (95% CI 24-62%)	-	25	(34)
CCG-1953	97%	5 yr	42% (9%)	45% (6%)	115	(59)
CCG-1883	97%	4 yr	39% (4%)	51% (4%)	135	(29)
CCG-107	94%	4 yr	33% (5%)	45% (5%)	99	(29)
UKALL-92	94%	5 yr	33% (95% CI 23-44%)	46% (95% CI 35-57%)	86	(60)
POG 8493	93%	4 yr	28% (5%)	-	82	(32)
POG alternating drugs	94%	4 yr	17% (8%)	-	33	(31)

CR = complete remission; EFS = event- free survival

Comparisons of different treatment protocols and outcome are difficult because most protocols differ in many details and the reported patient numbers are often low.

A small study by several POG institutions, resulted in a 5-year EFS of only 17%. (31). Unlike other protocols, this regimen did not contain dexamethasone, high-dose methotrexate (MTX), high-dose ara-c, cyclophosphamide or ifosfamide whereas L-asparaginase was used in the induction phase only. In another POG study (32) the EFS rate was 27%, which is also lower than the results of other study groups. This protocol lacked dexamethasone, L-asparaginase, anthracyclines, high-dose

ara-c and high-dose MTX. Protocols of MRC UKALL specified high-dose MTX dose and high-dose ara-c, but not dexamethasone, cyclophosphamide or ifosfamide. (33) L-asparaginase was administered only in the induction phase. The overall EFS rate was only 25%.

The Dana-Farber Cancer Institute (DFCI) consortium intensified its treatment protocols since 1985. This led to a significant improvement for infants with an EFS of 54% in a very small series of cases (28). The main difference with the historical control series was the use of a postinduction intensification course with high-dose MTX, high-dose ara-c, L-asparaginase, vincristine and 6-mercaptopurine. Dexamethasone, cyclophosphamide or ifosfamide, and epipodophyllotoxins were all excluded from this DFCI protocol but cranial irradiation was administered at the age of 1 year.

The CCG-1883 resulted in 39% EFS (24) which was higher than historical CCG control series in which less intensive systemic therapies were used. Major difference was the inclusion of high-dose ara-c, cyclophosphamide, and more L-asparaginase in the consolidation and reconsolidation phases. An important finding was that intensive chemotherapy combined with intrathecal therapy result in the same CNS relapse rate as earlier schedules including cranial irradiation, even in patients with CNS involvement at initial diagnosis (29). In particular, high-dose MTX, high-dose ara-c, dexamethasone, and intrathecal therapy may have contributed to reduction of CNS relapses.

Since 1983, BFM investigators stratified patients according to the prednisone response and leukemic cell burden, resulting in treatment of infants according to different arms of the protocols. In general, infants were over-represented in the higher-risk arms because of their high leukemic burden and high incidence of poor prednisone response. The overall EFS rate for infants on BFM protocols was 43% (21). Small studies of the EORTC–CLG (34) and AIEOP (35) that also used BFM regimens reached 43% and 45% EFS respectively. In these BFM based protocols, cranial irradiation was given to a subgroup of the patients and 30-60% of the infants were not treated by “regular” ALL therapy but by intensive high-risk chemotherapy courses of BFM (21), (34), (35). The 47% EFS achieved by the intergroup Interfant-99 study is comparable to the best reported outcomes in single group studies but Interfant protocol did not include intensive high risk courses such as in BFM protocols. Interfant-99 is based on a regular ALL protocol with addition of araC in different doses and schedules. On Interfant-99, prednisone-good responders had a similar outcome as reported by the BFM study whereas prednisone-poor responders obtained a 30% EFS compared with 15% in the BFM study (21), (34), (35). In Interfant-99 no irradiation was used, no alkylating agents and a low dose of anthracyclines was used and very few patients received BMT.

It is worth to mention the outcome of patients in the first month of life, socalled congenital ALL because this is relatively often assumed to be fatal. No studies had been published on this except for case reports (36). The Interfant-99 study included 30 patients with congenital ALL for whom the 2-year event-free survival and survival appeared to be 20%. Early death in complete remission and treatment delays resulting from toxicity were not different form that in older infants. The survival of 17% after last follow-up, combined with a toxicity profile comparable with that in older infants, justifies treating congenital ALL with curative intent (37).

Bone marrow transplantation (BMT). No randomized studies have compared allogeneic BMT with chemotherapy; many small (single institution) and biased series have been published. A meta-analysis (38) did not show a benefit for the use of allogenic BMT from a matched donor in infant MLL gene rearranged ALL. The combined results of two consecutive Japanese studies using intensive chemotherapy blocks followed by BMT in case of MLL rearrangement resulted in a long-term EFS of 50% (39). This regimen resulted however in a significant number of serious late effects. Also, 8 out of 53 patients who underwent BMT died from toxicity and over half of the events occurred before instigation of BMT.

The Interfant-99 study did not show a significant benefit of BMT for prednisone poor responders (4) but more recent analyses indicates that high-

risk patients as currently defined in the Interfant-06 protocol, benefited from the use of BMT in the Interfant-99 protocol. This high risk group is defined by the presence of all 3 of the following risk criteria: (a) MLL rearrangement AND (b) age below 6 months at diagnosis AND (c) a poor prednisone response or a WBC >300x10e9/L (40). If patients did not fulfil all 3 criteria the outcome was not different between patients that received chemotherapy only or chemotherapy followed by BMT.

So, in conclusion, Interfant, BFM based regimens and Japanese protocols have achieved the best outcome results. The BFM strategy implied the use of intensive high risk courses for a large number of infants and the Japanese approach implied BMT for almost all MLL rearranged cases with substantial morbidity and mortality. In general, we can conclude that intensive postinduction chemotherapy and the use of high-dose ara-c, high-dose MTX, L-asparaginase, dexamethasone and cyclophosphamide or ifosfamide are probably helpful in preventing early bone marrow relapses. BMT should be reserved for a small group of selected high risk cases

Late effects

Little is known about late effects of treatment for infant ALL, mainly because substantial numbers of infants did not survive until recently. Learning disabilities and developmental delays were identified in the majority of irradiated infants (28), (34). Obesity and short stature were found in ~25% of irradiated cases. Asymptomatic echocardiographic abnormalities and stable congestive heart failure have been reported in single cases. (28), (34). In 30 nonirradiated infants who were treated with high-dose MTX as CNS-directed therapy, the neurodevelopmental outcome was normal (41). Frankel (32) reported on one patient with a severe developmental disorder among 18 infants who were neither irradiated nor transplanted and remained in complete remission. The Japanese study group did not observe significant late effects in patients who did not receive BMT. However, several serious late complications were seen in a substantial proportion of patients who did receive BMT such as chronic graft versus host disease, hypothyroidism, skin abnormalities, ophthalmologic complications, pulmonary complications, dental abnormalities and neurocognitive problems (39). As treatment has become more effective for infants with leukemia nowadays, it is important to incorporate prospective late effects analyses.

Drug Dosage Adjustment and pharmacokinetics

A persistent problem are the rules for drug dosage adjustment in infants (42). In general, the total-body water content decreases from 75% at birth to 60% at 1 year, and the percentage of extracellular water decreases with age. Drugs bind less avidly to serum proteins in newborns than in adults, leading to a higher unbound active fraction of drugs in infants. The lower activity of P-450 enzymes in infants can lead to reduced cytotoxic effects as well as increased cytotoxic effects. Drugs cleared by the kidneys may have increased systemic exposures in young infants because tubular and glomerular function reach adult levels by ~6 months of age (42). The volume of the CNS relative to body surface area or body weight, is larger in children compared to adults. Therefore, intrathecal chemotherapy should be calculated on age and not on body surface to avoid undertreatment of infants (43). The ratio of body weight to body surface is lower in infants than in older children, which implies that if dosages are calculated on body weight, infants are exposed to lower amounts of drugs.

Three studies have looked at MTX pharmacokinetics in infants. The first and small study showed no decreased clearance of MTX in infants compared to older children (44) whereas a more recent report showed that MTX clearance was slightly lower in younger infants (0-6 months) than in older infants (7-12 months). Steady-state clearance for these older infants appeared to be comparable to values reported for older children. Very young infants (0-3 months) experienced a slightly higher incidence of renal toxicity but no difference in liver toxicity or mucositis (45). Very recently, the Interfant collaborative group reported on 103 infants at the time of their first treatment with methotrexate (5 g/m/2) (46). In the Interfant-99 protocol, infants <6 months of age received two-third, children 6-12 months three-fourth, and children >12 months full dose calculated on body surface area

(BSA). The systemic clearance tended to increase with age. All infants tolerated the dose well enough to receive a second dose of MTX without further dose reduction. No significant effect on disease-free survival for MTX steady-state concentration, MTX clearance, or time to MTX level below 0.2 microM was found. Interestingly, male infants had higher clearance than female infants. So, younger infants have slightly lower MTX clearance than older infants and when using dose reduction rules as applied in Interfant-99, this leads to a comparable toxicity profile as for older infants. However, in view of the poor treatment results for especially young infants, one might also consider not to decrease the dose for these patients or to increase the dose for those who reach low plasma levels after the first MTX dose (46).

Hempel et al showed (47) daunorubicin clearance, central volume of distribution, apparent clearance of daunorubicinol and apparent volume of distribution showed no age-dependency. Consequently, due to the empirical dose reduction in Interfant-99 the overall exposure to daunorubicinol in infants was smaller than would be expected from older children. Patients aged <6 months experienced more infections in the induction phase than the group aged 6-12 months at diagnosis. Other toxicities were similar in both groups. The authors concluded that there was no age-dependency in the pharmacokinetics of daunorubicin

It has been suggested that infants show decreased ara-C clearance after high-dose therapy with this agent because of poorer conversion of ara-C to ara-U (48). Others have not found a difference in ara-C clearance between infants and older children (49).

In general, pharmacokinetic studies in infants are very scarce while many protocols rely on arbitrary calculations based on body weight, body surface area or one of these in combination with arbitrary dose reductions by age. Thus, pharmacokinetic studies together with toxicity measurements are urgently needed in infants.

New therapeutic strategies

Combinations of multiple new drugs will be required to cure infant MLL gene rearranged ALL patients who are not cured with current chemotherapies. Thus, innovative strategies are needed that either overcome resistance to conventional drugs or which involve alternative novel agents that more effectively target infant MLL cells (16).

Given the sensitivity of infant ALL cells to nucleoside analogues such as araC and 2CdA as described above, newly developed nucleoside analogues may be interesting candidate drugs for further analysis in infant ALL. Clofarabine has been shown to be effective in refractory or relapsed ALL in childhood and is also transported by the ENT1 protein. So it seems worthwile to investigate this drug in infant ALL.

Another class of drugs that may be effective against MLL gene rearranged ALL cells are demethylating cytidine analogues, such as 5-azacytidine, 5-aza-2'-deoxycytidine (decitabine), or the recently identified agent zebularine. As mentioned above, especially t(4;11) and t(11;19) positive ALL are characterised by aberrant DNA hypermethylation. The degree of hypermethylation may influence outcome in infant ALL and that demethylating agents largely reverses the aberrant methylation pattern of MLL rearranged ALL cells, leading to apoptosis in these cells (50). In concordance with this, we observed that the tumour suppressor gene *FHIT* was silenced by methylation of the promoter region in 100% of the infant MLL gene rearranged cases tested, whereas silencing of this gene was observed in only 50% of older children with ALL (51). Ectopic expression of *FHIT* in MLL rearranged cells induced leukaemic cell death. Likewise, treatment with the demethylating agent decitabine resulted in re-expression of FHIT protein expression and induced apoptosis. In conclusion, inhibition of DNA methylation may be an effective therapeutic strategy in the treatment of infant MLL, especially since several demethylating agents also depend on ENT1 to cross the cell membrane, which is highly expressed in infant ALL cells (27).

FLT3, the gene encoding Fms-like tyrosine kinase 3, is highly expressed in patients with MLL gene rearranged ALL (8). FLT3 is important in early B-lineage development and is highly expressed in immature B-cells (52). In AML the *FLT3* gene is frequently subjected to mutations

that activate this receptor (53). Constitutively activated FLT3 became a promising therapeutic target in AML and several small molecule inhibitors (e.g. CEP-701, PKC412 and SU5416) inactivate FLT3 and induce leukemic cell death. This has led to the initiation of clinical trials with these inhibitors in adult AML, and so far the results are promising. Interestingly, constitutively activated FLT3 also occurs in MLL rearranged infant ALL patients carrying activating mutations, and in MLL rearranged infant ALL displaying high-level expression of wild-type *FLT3* (26), (54). We and others demonstrated that high-level wild-type *FLT3* expression in primary infant MLL rearranged ALL samples is associated with activated FLT3 and cytotoxic responsiveness to FLT3 inhibitors (55), (56). Also, the level of FLT3 expression has prognostic relevance (57). This showed that FLT3 inhibition represents a novel therapeutic strategy for infant MLL which has led to two ongoing clinical trials exploring this.

Infant ALL has myeloid characteristics as mentioned above and the fact that MLL stands for mixed lineage leukemia illustrates that the leukemic cells in which the MLL gene gets affected are very immature and may differentiate into different lineages or have biphenotypic features. Chemotherapy blocks as being used for acute myeloid leukemia may have value in infant ALL therefore. This is currently being explored in the Interfant-06 protocol which compares the use of two AML induction courses versus protocol IB of the BFM protocol after induction therapy.

Several studies in children and adults with ALL have shown that minimal residual disease (MRD) status is a strong prognostic factor. Data in infants are scarce. Very recently we evaluated the prognostic significance of MRD in ~100 cases of infant ALL (58). All patients with MRD levels $>/=10^{-4}$ after consolidation relapsed. These patients are now eligible for BMT in the current Interfant-06 protocol.

Conclusions

Infant ALL showes a highly unfavorable outcome compared to that of older children with this disease subtype, which possesses unique clinical and biologic features. The major problem in treatment is the occurrence of early relapses, justifying early intensive chemotherapy whereas only a small selected subgroup of high risk patients may benefit from allogenic BMT. Large collaborative studies are the only way to investigate possible improvements of therapy for infants with ALL. New insights in the biology of MLL rearranged ALL have suggested new innovative approaches which will be tested in real life now and in the near future in an attempt to increase the cure rate to the same rate as that in older children with ALL.

References

1. Pieters R. Biology and treatment of infant leukemias: Humana Press; 2003.
2. Gale KB, Ford AM, Repp R, Borkhardt A, Keller C, Eden OB, et al. Backtracking leukemia to birth: identification of clonotypic gene fusion sequences in neonatal blood spots. Proc Natl Acad Sci U S A. 1997 Dec 9;94(25):13950-4.
3. Ridge SA, Cabrera ME, Ford AM, Tapia S, Risueno C, Labra S, et al. Rapid intraclonal switch of lineage dominance in congenital leukaemia with a MLL gene rearrangement. Leukemia. 1995 Dec;9(12):2023-6.
4. Pieters R, Schrappe M, De Lorenzo P, Hann I, De Rossi G, Felice M, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. Lancet. 2007 Jul 21;370(9583):240-50.
5. Jansen MW, Corral L, van der Velden VH, Panzer-Grumayer R, Schrappe M, Schrauder A, et al. Immunobiological diversity in infant acute lymphoblastic leukemia is related to the occurrence and type of MLL gene rearrangement. Leukemia. 2007 Apr;21(4):633-41.
6. van der Burg M, Beverloo HB, Langerak AW, Wijsman J, van Drunen E, Slater R, et al. Rapid and sensitive detection of all types of MLL gene translocations with a single FISH probe set. Leukemia. 1999 Dec;13(12):2107-13.
7. Dou Y, Hess JL. Mechanisms of transcriptional regulation by MLL and its disruption in acute leukemia. Int J Hematol. 2008 Jan;87(1):10-8.
8. Armstrong SA, Staunton JE, Silverman LB, Pieters R, den Boer ML, Minden MD, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. Nat Genet. 2002 Jan;30(1):41-7.
9. Yeoh EJ, Ross ME, Shurtleff SA, Williams WK, Patel D, Mahfouz R, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell. 2002 Mar;1(2):133-43.
10. Stam RW, Schneider P, Hagelstein JA, van der Linden MH, Stumpel DJ, de Menezes RX, et al. Gene expression profiling-based dissection of MLL translocated and MLL germline acute lymphoblastic leukemia in infants. Blood. 2010 Apr 8;115(14):2835-44.
11. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. Nat

Rev Cancer. 2007 Nov;7(11):823-33.

12. Baylin SB, Esteller M, Rountree MR, Bachman KE, Schuebel K, Herman JG. Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. Hum Mol Genet. 2001 Apr;10(7):687-92.
13. Schotte D, Chau JC, Sylvester G, Liu G, Chen C, van der Velden VH, et al. Identification of new microRNA genes and aberrant microRNA profiles in childhood acute lymphoblastic leukemia. Leukemia. 2009 Feb;23(2):313-22.
14. Guenther MG, Jenner RG, Chevalier B, Nakamura T, Croce CM, Canaani E, et al. Global and Hox-specific roles for the MLL1 methyltransferase. Proc Natl Acad Sci U S A. 2005 Jun 14;102(24):8603-8.
15. Schotte D, Lange-Turenhout EA, Stumpel DJ, Stam RW, Buijs-Gladdines JG, Meijerink JP, et al. Expression of miR-196b is not exclusively MLL-driven but especially linked to activation of HOXA genes in pediatric acute lymphoblastic leukemia. Haematologica. 2010 May 21.
16. Stam RW, den Boer ML, Pieters R. Towards targeted therapy for infant acute lymphoblastic leukaemia. Br J Haematol. 2006 Mar;132(5):539-51.
17. Kumagai M, Manabe A, Pui CH, Behm FG, Raimondi SC, Hancock ML, et al. Stroma-supported culture in childhood B-lineage acute lymphoblastic leukemia cells predicts treatment outcome. J Clin Invest. 1996 Feb 1;97(3):755-60.
18. Uckun FM, Sather H, Reaman G, Shuster J, Land V, Trigg M, et al. Leukemic cell growth in SCID mice as a predictor of relapse in high-risk B-lineage acute lymphoblastic leukemia. Blood. 1995 Feb 15;85(4):873-8.
19. Kersey JH, Wang D, Oberto M. Resistance of t(4;11) (MLL-AF4 fusion gene) leukemias to stress-induced cell death: possible mechanism for extensive extramedullary accumulation of cells and poor prognosis. Leukemia. 1998 Oct;12(10):1561-4.
20. Pieters R, den Boer ML, Durian M, Janka G, Schmiegelow K, Kaspers GJ, et al. Relation between age, immunophenotype and in vitro drug resistance in 395 children with acute lymphoblastic leukemia—implications for treatment of infants. Leukemia. 1998 Sep;12(9):1344-8.
21. Dordelmann M, Reiter A, Borkhardt A, Ludwig WD, Gotz N, Viehmann S, et al. Prednisone response is the strongest predictor of treatment outcome in infant acute lymphoblastic leukemia. Blood. 1999 Aug 15;94(4):1209-17.
22. Stam RW, Den Boer ML, Schneider P, de Boer J, Hagelstein J, Valsecchi MG, et al. Association of high-level MCL-1 expression with in vitro and in vivo prednisone resistance in MLL-rearranged infant acute lymphoblastic leukemia. Blood. 2010 Feb 4;115(5):1018-25.
23. Wei G, Twomey D, Lamb J, Schlis K, Agarwal J, Stam RW, et al. Gene expression-based chemical genomics identifies rapamycin as a modulator of MCL1 and glucocorticoid resistance. Cancer Cell. 2006 Oct;10(4):331-42.
24. Stam RW, van den Heuvel-Eibrink MM, den Boer ML, Ebus ME, Janka-Schaub GE, Allen JD, et al. Multidrug resistance genes in infant acute lymphoblastic leukemia: Ara-C is not a substrate for the breast cancer resistance protein. Leukemia. 2004 Jan;18(1):78-83.
25. Ramakers-van Woerden NL, Beverloo HB, Veerman AJ, Camitta BM, Loonen AH, van Wering ER, et al. In vitro drug-resistance profile in infant acute lymphoblastic leukemia in relation to age, MLL rearrangements and immunophenotype. Leukemia. 2004 Mar;18(3):521-9.
26. Stam RW, Hubeek I, den Boer ML, Buijs-Gladdines JG, Creutzig U, Kaspers GJ, et al. MLL gene rearrangements have no direct impact on Ara-C sensitivity in infant acute lymphoblastic leukemia and childhood M4/M5 acute myeloid leukemia. Leukemia. 2006 Jan;20(1):179-82.
27. Stam RW, den Boer ML, Meijerink JP, Ebus ME, Peters GJ, Noordhuis P, et al. Differential mRNA expression of Ara-C-metabolizing enzymes explains Ara-C sensitivity in MLL gene-rearranged infant acute lymphoblastic leukemia. Blood. 2003 Feb 15;101(4):1270-6.
28. Silverman LB, McLean TW, Gelber RD, Donnelly MJ, Gilliland DG, Tarbell NJ, et al. Intensified therapy for infants with acute lymphoblastic leukemia: results from the Dana-Farber Cancer Institute Consortium. Cancer. 1997 Dec 15;80(12):2285-95.
29. Reaman GH, Sposto R, Sensel MG, Lange BJ, Feusner JH, Heerema NA, et al. Treatment outcome and prognostic factors for infants with acute lymphoblastic leukemia treated on two consecutive trials of the Children's Cancer Group. J Clin Oncol. 1999 Feb;17(2):445-55.
30. Ludwig WD, Rieder H, Bartram CR, Heinze B, Schwartz S, Gassmann W, et al. Immunophenotypic and genotypic features, clinical characteristics, and treatment outcome of adult pro-B acute lymphoblastic leukemia: results of the German multicenter trials GMALL 03/87 and 04/89. Blood. 1998 Sep 15;92(6):1898-909.
31. Lauer SJ, Camitta BM, Leventhal BG, Mahoney D, Jr., Shuster JJ, Kiefer G, et al. Intensive alternating drug pairs after remission induction for treatment of infants with acute lymphoblastic leukemia: A Pediatric Oncology Group Pilot Study. J Pediatr Hematol Oncol. 1998 May-Jun;20(3):229-33.
32. Frankel LS, Ochs J, Shuster JJ, Dubowy R, Bowman WP, Hockenberry-Eaton M, et al. Therapeutic trial for infant acute lymphoblastic leukemia: the Pediatric Oncology Group experience (POG 8493). J Pediatr Hematol Oncol. 1997 Jan-Feb;19(1):35-42.
33. Chessells JM, Eden OB, Bailey CC, Lilleyman JS, Richards SM. Acute lymphoblastic leukaemia in infancy: experience in MRC UKALL trials. Report from the Medical Research Council Working Party on Childhood Leukaemia. Leukemia. 1994 Aug;8(8):1275-9.
34. Ferster A, Bertrand Y, Benoit Y, Boilletot A, Behar C, Margueritte G, et al. Improved survival for acute lymphoblastic leukaemia in infancy: the experience of EORTC-Childhood Leukaemia Cooperative Group. Br J Haematol. 1994 Feb;86(2):284-90.
35. Biondi A, Rizzari C, Valsecchi MG, De Lorenzo P, Arico M, Basso G, et al. Role of treatment intensification in infants with acute lymphoblastic leukemia: results of two consecutive AIEOP studies. Haematologica. 2006 Apr;91(4):534-7.

36. Bresters D, Reus AC, Veerman AJ, van Wering ER, van der Does-van den Berg A, Kaspers GJ. Congenital leukaemia: the Dutch experience and review of the literature. Br J Haematol. 2002 Jun;117(3):513-24.

37. van der Linden MH, Valsecchi MG, De Lorenzo P, Moricke A, Janka G, Leblanc TM, et al. Outcome of congenital acute lymphoblastic leukemia treated on the Interfant-99 protocol. Blood. 2009 Oct 29;114(18):3764-8.

38. Pui CH, Gaynon PS, Boyett JM, Chessells JM, Baruchel A, Kamps W, et al. Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet. 2002 Jun 1;359(9321):1909-15.

39. Tomizawa D, Koh K, Sato T, Kinukawa N, Morimoto A, Isoyama K, et al. Outcome of risk-based therapy for infant acute lymphoblastic leukemia with or without an MLL gene rearrangement, with emphasis on late effects: a final report of two consecutive studies, MLL96 and MLL98, of the Japan Infant Leukemia Study Group. Leukemia. 2007 Nov;21(11):2258-63.

40. Mann G, Attarbaschi A, Schrappe M, De Lorenzo P, Peters C, Hann I, et al. Improved outcome with hematopoietic stem cell transplantation in a poor prognostic subgroup of infants with mixed-lineage-leukemia (MLL)-rearranged acute lymphoblastic leukaemia: results from the Interfant-99 Study. Blood. 2010 Jun 30.

41. Kaleita TA, Reaman GH, MacLean WE, Sather HN, Whitt JK. Neurodevelopmental outcome of infants with acute lymphoblastic leukemia: a Children's Cancer Group report. Cancer. 1999 Apr 15;85(8):1859-65.

42. Biondi A, Cimino G, Pieters R, Pui CH. Biological and therapeutic aspects of infant leukemia. Blood. 2000 Jul 1;96(1):24-33.

43. Bleyer AW. Clinical pharmacology of intrathecal methotrexate. II. An improved dosage regimen derived from age-related pharmacokinetics. Cancer Treat Rep. 1977 Nov;61(8):1419-25.

44. Donelli MG, Zucchetti M, Robatto A, Perlangeli V, D'Incalci M, Masera G, et al. Pharmacokinetics of HD-MTX in infants, children, and adolescents with non-B acute lymphoblastic leukemia. Med Pediatr Oncol. 1995 Mar;24(3):154-9.

45. Thompson PA, Murry DJ, Rosner GL, Lunagomez S, Blaney SM, Berg SL, et al. Methotrexate pharmacokinetics in infants with acute lymphoblastic leukemia. Cancer Chemother Pharmacol. 2007 May;59(6):847-53.

46. Lonnerholm G, Valsecchi MG, De Lorenzo P, Schrappe M, Hovi L, Campbell M, et al. Pharmacokinetics of high-dose methotrexate in infants treated for acute lymphoblastic leukemia. Pediatr Blood Cancer. 2009 May;52(5):596-601.

47. Hempel G, Relling MV, de Rossi G, Stary J, De Lorenzo P, Valsecchi MG, et al. Pharmacokinetics of daunorubicin and daunorubicinol in infants with leukemia treated in the interfant 99 protocol. Pediatr Blood Cancer. 2010 Mar;54(3):355-60.

48. Periclou AP, Avramis VI. NONMEM population pharmacokinetic studies of cytosine arabinoside after high-dose and after loading bolus followed by continuous infusion of the drug in pediatric patients with leukemias. Cancer Chemother Pharmacol. 1996;39 (1-2):42-50.

49. McLeod HL, Relling MV, Crom WR, Silverstein K, Groom S, Rodman JH, et al. Disposition of antineoplastic agents in the very young child. Br J Cancer Suppl. 1992 Aug; 18:S23-9.

50. Stumpel D, Schneider P, van Roon E, JM B, De Lorenzo P, Valsecchi MG, et al. Specific promoter methlation identifies different subgroups of MLL-rearranged infant Acute Lymphoblastic Leukemia, influences clinical outcome and provides therapeutic options. submitted. Stumpel DJ, Blood 2009;114: 5490-5498.

51. Stam RW, den Boer ML, Passier MM, Janka-Schaub GE, Sallan SE, Armstrong SA, et al. Silencing of the tumor suppressor gene FHIT is highly characteristic for MLL gene rearranged infant acute lymphoblastic leukemia. Leukemia. 2006 Feb;20(2):264-71.

52. Stirewalt DL, Radich JP. The role of FLT3 in haematopoietic malignancies. Nat Rev Cancer. 2003 Sep;3(9):650-65.

53. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. Blood. 2002 Sep 1;100(5):1532-42.

54. Armstrong SA, Kung AL, Mabon ME, Silverman LB, Stam RW, Den Boer ML, et al. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. Cancer Cell. 2003 Feb;3(2):173-83.

55. Stam RW, den Boer ML, Schneider P, Nollau P, Horstmann M, Beverloo HB, et al. Targeting FLT3 in primary MLL-gene-rearranged infant acute lymphoblastic leukemia. Blood. 2005 Oct 1;106(7):2484-90.

56. Brown P, Levis M, Shurtleff S, Campana D, Downing J, Small D. FLT3 inhibition selectively kills childhood acute lymphoblastic leukemia cells with high levels of FLT3 expression. Blood. 2005 Jan 15;105(2):812-20.

57. Stam RW, Schneider P, de Lorenzo P, Valsecchi MG, den Boer ML, Pieters R. Prognostic significance of high-level FLT3 expression in MLL-rearranged infant acute lymphoblastic leukemia. Blood. 2007 Oct 1;110(7):2774-5.

58. Van der Velden VH, Corral L, Valsecchi MG, Jansen MW, De Lorenzo P, Cazzaniga G, et al. Prognostic significance of minimal residual disease in infants with acute lymphoblastic leukemia treated within the Interfant-99 protocol. Leukemia. 2009 Jun;23(6):1073-9.

59. Hilden JM, Dinndorf PA, Meerbaum SO, Sather H, Villaluna D, Heerema NA, et al. Analysis of prognostic factors of acute lymphoblastic leukemia in infants: report on CCG 1953 from the Children's Oncology Group. Blood. 2006 Jul 15;108(2):441-51.

60. Chessells JM, Harrison CJ, Watson SL, Vora AJ, Richards SM. Treatment of infants with lymphoblastic leukaemia: results of the UK Infant Protocols 1987-1999. Br J Haematol. 2002 May;117(2):306-14.

INTERNATIONAL SOCIETY
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Paediatric Regimens for Adolescent & Young Adults

André Baruchel

Abstract

The problem of the management of adolescents and young adults (AYA) with acute lymphoblastic leukemia (ALL) has progressively emerged, mainly in the last ten years.

After recognizing that the biology disease was not identical to childhood ALL, pediatric investigators have focussed their efforts in two directions: intensification of the treatment and comparison of their results with adult protocols. This fruitful collaboration has led to the firm conclusion that the more intensive pediatric protocols were also more effective despite indicating less bone marrow transplantations. The results were so appealing that either "pediatric inspired "or pediatric protocols including patients until adulthood have been generated with promising results. More biology is still needed to understand differences with childhood ALL. More clinical research is still needed to prevent short term and long term toxic events in AYA.

Introduction

The word adolescent derives from the Latin *adolescere*, which means 'to grow'. Not surprisingly, there is thus no precise definition of adolescence or young adulthood. Some dictionaries define adolescence arbitrarily as 'around 12–18 years in girls and 14–20 years in boys'. The Anglo-Saxon word 'teenager' encompasses the period from 13 to 19 years. The World Health Organisation (WHO) definition (1986; http://whqlibdoc.who.int/trs/WHO_TRS_731_fre.pdf) considers adolescents to be individuals aged 10–19 years.

Whatever the exact definition, adolescents with cancer or leukaemia are treated either by paediatric hemato-oncologists, or by adult haematologists or oncologists. Young adults are treated by the latters. The concept of adolescents and young adults (AYA) has emerged recently in the field of cancer, particularly in acute lymphoblastic leukaemia (ALL)

1. Cancer in adolescents: facts and general comments regarding their treatment

Cancer is the leading cause of non-accidental death in children and adolescents under the age of 20 years[1]. In this age range, one-third of cases involve adolescents between 15 and 20 years of age[1, 2]. Hodgkin's and non-Hodgkin's lymphomas account for 25% of the tumours. Leukemias represent only 15% of all the tumours, compared with 30% prior to 10 years of age. ALL and acute myeloid leukaemia (AML) represent 65% and 35%, respectively, of all acute leukemias observed in the 15–20 years population versus 85% and 15%, respectively, in children under 15 years of age[2]. A small increase in cancers in this age range has been found in industrialised countries, essentially due to an apparent increase in ALLs[1].

1.1. Do adolescents benefit from the most adapted therapies?

Hemato-Oncology co-operative groups offer the best therapeutic options. This is demonstrated particularly in the paediatric setting[3]. One epidemiological problem is that only the patients included in protocols are registered. A large study in the United States of America (USA) has shown that 97.6% of the children aged 15 years or less are registered in Pediatric Oncology protocols, compared with only 21% of the adolescents aged 16–21 years[4]. In the latter category, less than 3% are registered in adult haematology or oncology protocols[4, 5]. Potential explanations are numerous, but this leads to the conclusion that a great part of the adolescent population is treated suboptimally, outside paediatric or adult haemato-oncology networks.

Even though these numbers probably do not represent the European reality, the same conclusion is at least partially applicable. A comparison of adult and paediatric therapeutic strategies in ALL will be detailed in paragraph 2.2.

1.2. Compliance and adolescents

One of the general problems encountered in treating a severe disease in an adolescent population is the diminished compliance to treatment. Some studies have documented this notion, sometimes by measuring the urinary or serum level of the prescribed drugs. Festa and colleagues have thus evaluated compliance with prednisone treatment in adolescents treated for ALL and Hodgkin's disease: 52% of the patients were considered to be non-adherent to the treatment[6]. A nationwide study in the United Kingdom (UK) of intracellular drug metabolite concentrations in 496 children who had been prescribed 6-mercaptopurine for the treatment of ALL was carried out to assess inter-patient variability at a standardised dose. Nine children (2% of the total) had completely undetectable metabolites, indicative of complete non-compliance, five of whom were adolescents[7]. Numerous factors seem to influence compliance, including socio-economic status, comprehension of the mode of drug administration, easiness of drug availability, clear definition of the responsibilities of the adolescent and his or her parents, and the number of children in the family[8]. Further research on this subject is underway.

2. Acute lymphoblastic leukaemia in adolescents

2.1. Prognostic parameters

Five-year event-free survival (EFS) of children with ALL is now exceeding 80%[9]. Age is a well-known prognostic variable. A classic age limit is set at 10 years, as used in the Rome–National Cancer Institute (NCI) classification[10]. Nevertheless, this limit is rather 'fuzzy', some teams finding a worst prognosis after 6 or 7 years[11,12], other groups considering a limit of 11 years as relevant[13]. In fact it seems that after the peak of common ALL a progressive decrease in the prognosis is observed, leading to the worst prognosis of adult ALL (approximately 30–60% cure rate)[14].

Adolescents over 15 years of age have been known to have a poorer prognosis, resembling the one of young adults, in terms of obtaining a complete remission (CR)[13] or disease-free survival (DFS) duration[13–16]. Two studies from the Memphis group, performed in the 1980s, showed a significant difference in outcome between the children aged from 10 to 15 years and adolescents above 15 years [15,16]. The current view is that this is now not the case, as demonstrated by several recent studies, favouring the idea that adolescence begins at 10 years in ALL. A Children's Cancer Group study shows identical EFS for the two subpopulations, but inferior EFS to the one in those patients under 10 years old[17]. The same observation has been made for patients treated within the French Acute Lymphoblastic Leukaemia Group (FRALLE) 93 protocol (5y EFS of the 10-14: 64±6% vs 68±11% for the 15-19, p=NS). The BFM group also made the same observation, particularly in the B-lineage ALLs ((10y EFS of the 10-14: 60.6±2.9% vs 63.7±5.3% for the 15-18, p=NS)[18]. A study from the Dana Farber Cancer Institute conducted between 1991 and 2000 has also been published recently[19]. The authors compared the outcomes in three age groups: children aged 1–10 years (n = 685), young adolescents aged 10–15 years (n = 108), and older adolescents aged 15–18 years (n = 51). With a median follow-up of 6.5 years, the 5-year EFS for those aged 1–10 years was 85% (standard error (SE) 1%), compared with 77% (SE 4%) for those aged 10–15 years, and 78% (SE, 6%) for those aged 15–18 years (P = 0.09)[19].

Reasons associated with a worse prognosis in adolescents are multifactorial:

2.1.1. Factors linked to the patient

More boys than girls are encountered in this group, male gender being associated with a worse prognosis.

The pharmacological characteristics of this population are not well known. Nevertheless, the toxicity of some major drugs for ALL is augmented, leading to dose reduction. For example, adolescents have a diminished clearance of vincristine compared with younger children (under 10 years of age), explaining the neurotoxicities observed[20]. A greater frequency

of avascular necrosis (AVN) is encountered with dexamethasone. Burger and colleagues retrospectively analysed 1951 patients under 18 years of age, who were treated according to trial ALL-BFM 95 between 1996 and 2000. The overall 5-year cumulative incidence for AVN is 1.8%. The incidence for patients < 10 years is 0.2%, whereas for patients = 10 years it is 8.9% ($P = 0.001$) and for patients = 15 years and less than 19 years it is 16.7% ($P = 0.003$)[21]. Similarly, in the recently published CCG 1961 study a 19.9% incidence has been reported for the 16-21 year-old age group[22]. A higher risk of central nervous system (CNS) thrombosis linked to L-asparaginase has been suggested in girls using contraception.

Reduced compliance is likely to interfere with the intensity of oral maintenance treatment with mercapto-purine and methotrexate, the paramount importance of which has been well-established[23].

2.1.2. Features linked to the disease

Beyond the age of 10 years are encountered ALLs carrying a higher risk of treatment failure. A summary of these features is given in Table 1.

Table 1. Biological features often encountered in adolescents with acute lymphoblastic leukaemia (ALL)

WBC count > 50,000 /mm^3
Elevated LDH
T-cell ALL
B-lineage CD10-negative ALL
Low incidence of hyperdiploidy
Very low incidence of t(12;21)/TEL-AML1 positive ALL
Slight increase in Philadelphia-positive ALL
Increased deletions/mutations of IKZF1?
Increased overexpression of CRLF2?
Poor early response to prednisone

WBC, white blood cell; LDH, lactate dehydrogenase;

A clear increase in the T-cell ALL frequency is documented (less than 15% under 15 years of age compared with 20–30% above this age), a feature associated to a higher risk of failure. A cohort of 258 adolescents (15–20 years old) were treated in the successive FRALLE 83, FRALLE 87–89, FRALLE 92 (pilot phase), FRALLE 93 and FRALLE 2000 protocols (Baruchel ASH 06). The main characteristics were: a sex ratio of 1.8 (M/F), a B-lineage in 71% of cases versus T-lineage in 29% of patients aged 15–20 years between 1987 and 1999 with 27% of T-ALL (Baruchel ASH 06). Nachman and colleagues report a 21% incidence in 143 adolescents aged 16–21 years[17]. These numbers are the same as those encountered in the adult population[14]. A progressive increase in B-lineage Philadelphia chromosome positive ALL, associated with a dismal prognosis has been reported after the age of 15 years, and particularly over the age of 20 years. No such an observation has been made in the FRALLE/LALA) study, described below, on 177 patients aged 15–20 years (incidence: 2.5%)[24].

A lower incidence of forms associated with a good outcome is observed in that population: incidence of hyperdiploidy is reduced[13, 15, 16]. The frequency of hyperdiploidy more than 50 chromosomes was 16% in the recent FRALLE/LALA study, an intermediate value between the 25% observed in children and the 5% displayed by adults[24]. Only rare forms with TEL-AML1 leukaemia are observed above the age of 10 years. This cryptic t(12;21) rearrangement, observed in about 20% of cases of childhood ALL, but in less than 2% of cases of adult ALL, was present in 7% of adolescents in the FRALLE-93 trial[24]. Even if a rare event in childhood, ALL (2-3%) amplification of the long arm of chromosome 21 is more frequent in older children and adolescents and seems to be

associated with a worse prognosis[25, 26]. Finally the contribution of the recently described IKZF1 deletions/mutations and CRLF2 overexpression to the worse prognosis of adolescents is to be exactly quantified[27, 28].

The cytogenetic 'black hole', at the frontier between adult and childhood populations, suggests the existence of unknown factors to explain the worse prognosis of adolescents among children. It is hoped that current studies on genomics or proteomics will throw light on this issue.

Several studies have also reported differences in ALL cell sensitivity to corticosteroids and chemotherapy *in vitro* [29, 30]. No study detailing the early response in term of minimal residual disease and according to lineage is yet available in this population.

2.2. Paediatric or adult protocols?

Adolescents, considered as high-risk patients by paediatricians, are considered as good risk patients when evaluated by adult haematologists[31].

Paediatric protocols, which are generally much more intensive than adult protocols, give the best outcome, even if all the comparative studies are retrospective. After the first fully reported French study[24], numerous studies have confirmed that notion. They are summarised in Table 2[32-37, 45].

Table 2. Comparison of paediatric and adult trials including adolescents in their study population (modified and actualised from Ramanujachar and colleagues[45]

Trial	Years	Age range (years)	Adolescent age range (years)	*n*	CR rate (%)	EFS	DFS	OS
FRALLE 83 LALA 85	1983–87 1985–	0–20 15–60	15–20 15–20	48 31	89 87	– –	47.5 (6 years) 32 (4 years)	– –
FRALLE 93 LALA 94	1993–99 1994–2000	0–20 15–adult	15–20 15–20	77 100	94 83	67 (5 years) 41 (5 years)	72 (5 years) 49 (5 years)	78 (5 years) 45 (5 years)
CCG 1882,1901 CALGB	1989–95 1988–98	0–21 16–adult	16–20 16–20	197 124	90 90	63 (7 years) 34 (7 years)	– –	67 (7 years) 46 (7 years)
AIEOP ALL 95, 2000 GIMEMA ALL 0496, 2000	1996–2003 1996–2003	0–18 14–adult	14–18 14–18	150 95	94 89	– –	– –	80 (2 years) 71 (2 years)
DCOG 6-9 HOVON ALL-5, 18	1985–99 1985–99	0–18 15–adult	15–18 15–18	47 44	98 91	69 (5 years) 34 (5 years)	71 (5 years) 37 (5 years)	– –
NOPHO SAALLG	1992–2000 1994–2000	0–18 15–40	15–20 15–20	36 23	99 90	74 (5 years) 39 (5 years)	– –	– –
MRC ALL97/99 UKALL XII/E2993	1997–2002 1997–2002	0–17 15–55	15–17 15–17	61 67	98 94	65 (5 years) 49 (5 years)	– –	71 (5 years) 56 (5 years)

CR, complete remission after induction; EFS, event-free survival; DFS, disease-free survival; OS, overall survival; FRALLE, FRench Acute Lymphoblastic LEukemia group; LALA, Leucémies Aigues Lymphoblastiques de l'Adulte; CCG, Children's Cancer Group; CALGB, Cancer and Leukemia Group B; AIEOP, Associazone Italiana Ematologia ed Oncologia Pediatrica; GIMEMA, Grupo Italiano Malattie Ematologiche Maligne dell Adulte; DCOG, Dutch Childhood Oncology Group; HOVON, Dutch-Belgian Hemato-Oncology Cooperative Study Group; NOPHO, Nordic Society of Pediatric Hematology and Oncology; SAALLG, Swedish Adult ALL Group; MRC, Medical Research Council; UKALL, United Kingdom ALL study group.

We will first focus on the French report which was the only one to include all the individual data in the same database, allowing multivariate analysis[24]. From June 1993 and September 1994, 77 and 100 evaluable adolescents (= 15 years, < 20 years) were enrolled in the paediatric FRALLE-93 and adult LALA-94 protocols. Among the different prognostic factors, the trial was analysed for probability of achieving complete remission or EFS. Patients were younger in the FRALLE-93 (median age: 15.9 versus 17.9) but other characteristics were similar: median WBC

(18 versus 16 × 10^9/l), B/T-lineage (54/23 versus 72/28), CD10-negative (13% versus 15%), poor-risk cytogenetics (t(9;22), t(4;11), hypodiploidy < 45 chromosomes; 6% versus 5%). The CR rate depended on the white blood cell (WBC) count ($P = 0.005$) and the trial (94% versus 83%; $P = 0.04$). Univariate analysis showed that unfavourable prognostic factors for EFS were the WBC count ($P < 0.0001$), the trial (estimated 5-year EFS 67% versus 35%; $P < 0.0001$), T-lineage ($P = 0.01$) and cytogenetics ($P = 0.01$). Trial and WBC count remained significant parameters for EFS in multivariate analysis ($P < 0.0001$). Significant differences within the B-Cell-Precursor-ALL subgroup were also observed for achieving CR (98% versus 81%; $P = 0.002$) and EFS ($P = 0.0002$), and within the T-ALL subgroup for EFS ($P = 0.05$) in favour of the paediatric protocol. Age was not a significant prognostic factor in that population. The same feature was found in a previous study of 143 adolescents aged 16–21 years from the Children's Cancer Group, in which EFS for patients aged 16–17, 18–19 and 20 years did not differ significantly [17]. Disparities in drug administration and dose-intensity between protocols were looked for to explain these differences in outcome. Differences in induction courses, which could underlie the observed gain in CR rates, are essentially: (i) the continuous administration of higher doses of prednisone; and (ii) the use of L-asparaginase in the FRALLE-93 protocol. Few pharmacological data are available to explain further this difference in remission rates. However, the three times daily administration schedule of steroids was shown to be superior to a more spaced administration in paediatric ALL[38]. Moreover, a study by the Dana-Farber Cancer Institute demonstrated an improved response to increased dose of steroids in patients aged 1–18 years[39]. Considering protocol periods, higher doses of major drugs in the treatment of ALL were used in the paediatric protocol, within a shorter period of time (3 times more vincristine, 5 times more prednisone, 20 times more L-asparaginase in 26 months versus 30 months). In the recent study of the Dana-Farber Consortium, children aged 9–18 years may benefit from higher doses of L-asparaginase despite an increased related toxicity[40]. In patients with T-ALL, repeated doses of L-asparaginase during early treatment significantly improved outcome in a randomised study of the Pediatric Oncology Group[41].

The US report has compared data in the 16-20 age range from the CCG studies (197 pts, studies 1882 and 1901, 1989-95) and the CALGB studies (124 pts, 5 studies, 1988-2001)[33]. The authors also found a difference in the median age (16 vs 19 years), meaning that the youngest patients were more likely to be treated in pediatric institutions. The 7-year EFS was also in favour of the pediatric protocols: 63% vs 34% overall even if an age effect was found for the patients treated in the CALGB studies with a better prognosis for the 16-17 compared to the 18-20[33].

Moreover, the paediatric delayed intensifications may contribute to improve outcome. The efficacy of this strategy, initially proposed by the Berlin-Frankfurt-Munster study group[42] has been confirmed by the Children's Cancer Group Study in children older than 10 years[43], with increased benefit of an augmented therapy including a double delayed intensifications in slow early responder patients[44]. The further intensification of the consolidation done in the CCG 1961 was proven to benefit to D7 rapid early responders : 5-year EFS of 81.8% (SE, 7%) vs 66.8% (SE, 6.7%) for standard therapy[22].

Finally, therapeutic attitudes can interfere with the concept of dose-intensity. Intervals between CR date time and day 1 of the first post-remission course were significantly longer in patients treated in the adult LALA-94 protocol, suggesting that dose-intensity could also be modulated by the usual inclination of physicians in adult centres to give patients time 'to get their breath back'.

3. Conclusion

The currently available comparative data encourage the inclusion of AYA in intensive paediatric protocols and the design of new trials, inspired of paediatric protocols, for the treatment of younger adults with ALL as recently proposed[46-48]. These protocols should include all modern stratifiers for therapeutics including MRD studies.

Immediate and long-term toxicity must be evaluated carefully and prospectively. Nevertheless, the toxicity profile of the paediatric

approach is also likely to be inferior to that of currently available adult protocols, which make greater use of bone marrow transplantation in first CR.

It can be also recommended that only those physicians who are trained in the complexities of the intensive management of ALL and participation in co-operative studies should be involved in the care of adolescents and young adults with this rare disease.

Acknowlegments: Marie-Françoise Auclerc, Nicolas Boissel, Sylvie Chevret

Conflict of interest statement

None declared.

References

1. *Cancer statistics review, 1973–1987*. NIH publication no.90-2789. Bethesda, MD: National Cancer Institute; June 1990.
2. Krailo MD, Bernstein L, Sullivan-Halley J, Hammond GD. Patterns of enrolment on cooperative group studies. An analysis of trends from the Los Angeles County Cancer Surveillance Program. *Cancer* 1993;**71**(suppl 10):3325–30.
3. Murphy SB. The national impact of clinical cooperative group trials for paediatric cancer. *Med Pediatr Oncol* 1995;**24**:279–80.
4. Bleyer WA, Tejeda H, Murphy SB, et al. National Cancer Clinical Trials: children have equal access, adolescent do not. *J Adolesc Health* 1997;**21**:366–73.
5. Reaman GH, Bonfiglio J, Krailo M, et al. Cancer in adolescents and young adults. *Cancer* 1993;**71**(suppl 10):3206–9.
6. Festa RS, Tamaroff MH, Chasalow F, Lanzkowsky P. Therapeutic adherence to oral medication regimens by adolescents with cancer. I. Laboratory assessment. *J Pediatr* 1992;**20**:807–11.
7. Lancaster D, Lennard L, Lilleyman JS. Profile of non-compliance in lymphoblastic leukaemia. *Arch Dis Child* 1997;**76**:365–6.
8. Pritchard MT, Butow PN, Stevens MM, Duley JA. Understanding medication adherence in pediatric acute lymphoblastic leukemia: a review. *J Pediatr Hematol Oncol* 2006;**28**(12):816–23.
9. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med* 2006;**354**(2):166–78.
10. Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. *J Clin Oncol* 1996;**14**:18–24.
11. Schaison G, Sommelet D, Bancillon A, et al. Treatment of acute lymphoblastic leukemia French protocol Fralle 83-87. *Leukemia* 1992;**6**(suppl 2):148–52.
12. Reiter A, Schrappe M, Ludwig WD, et al. Chemotherapy in 998 unselected childhood acute lymphoblastic leukemia patients. Results and conclusions of the multicenter trial ALL-BFM 86. *Blood* 1994;**84**:3122–33.
13. Crist W, Pullen J, Boyett J, et al. Acute lymphoid leukemia in adolescents: clinical and biologic features predict a poor prognosis – a Pediatric Oncology Group Study. *J Clin Oncol* 1988;**6**:34–43.
14. Gokbuget N, Hoelzer D. Treatment of adult acute lymphoblastic leukemia. *Hematology Am Soc Hematol Educ Program* 2006:133–41.
15. Santana VM, Dodge RK, Crist WM, et al. Presenting features and treatment outcome of adolescents with acute lymphoblastic leukemia. *Leukemia* 1990;**4**(2):87–90.
16. Rivera GK, Pui CH, Santana VM, et al. Progress in the treatment of adolescents with acute lymphoblastic leukemia. *Cancer* 1993;**71**(suppl 10):3400–5.
17. Nachman J, Sather HN, Buckley JD, et al. Young adults 16–21 years of age at diagnosis entered on Childrens Cancer Group acute lymphoblastic leukemia and acute myeloblastic leukemia protocols. Results of treatment. *Cancer* 1993;**71**(suppl 10):3377–85.
18. Pui CH, Schrappe M, Ribeiro RC, Niemeyer CM. Childhood and adolescent lymphoid and myeloid leukemia. Hematology Am Soc Hematol Educ Program. 2004:118-45.
19. Barry E, De Angelo DJ, Neuberg D et al. Favorable outcome for adolescents with acute lymphoblastic leukemia treated on Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium Protocols. *J Clin Oncol* 2007;**25**(7):813–9.
20. Crom WR, de Graaf SS, Synold T, et al. Pharmacokinetics of vincristine in children and adolescents with acute lymphocytic leukemia. *J Pediatr* 1994;**125**:642–9.
21. Burger B, Beier R, Zimmermann M et al. Osteonecrosis: a treatment related toxicity in childhood acute lymphoblastic leukemia (ALL) – experiences from trial ALL-BFM 95. *Pediatr Blood Cancer* 2005;**44**(3):220–5.
22. Nachman JB, La MK, Hunger SP et al. Young adults with acute lymphoblastic leukemia have an excellent outcome with chemotherapy alone and benefit from intensive post-induction treatment: a report from the children's oncology group. J Clin Oncol. 2009 Nov 1;27(31):5189-94.
23. Schmiegelow K, Pulczynska MK, Seip M. White cell count during maintenance chemotherapy for standard-risk childhood acute lymphoblastic leukemia: relation to relapse rate. *Pediatr Hematol Oncol* 1988;**5**:259–67.
24. Boissel N, Auclerc MF, Lheritier V, et al. Should adolescents with acute lymphoblastic leukemia be treated as old children or young adults? Comparison of the French FRALLE-93 and LALA-94 Trials. *J Clin Oncol* 2003;**21**:774–80.
25. Soulier J, Trakhtenbrot L, Najfeld V et al. Amplification of band q22 of chromosome 21, including AML1, in older children with acute lymphoblastic leukemia: an emerging

molecular cytogenetic subgroup. *Leukemia* 2003;**17**(8):1679–82.

26. Moorman AV, Richards SM, Robinson HM et al. Prognosis of children with acute lymphoblastic leukemia (ALL) and intrachromosomal amplification of chromosome 21 (iAMP21). *Blood* 2007;**109**(6):2327–30.
27. Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009 Jan 29;360(5):470-80.
28. Cario G, Zimmermann M, Romey R et al. Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. Blood. 2010 Apr 8.
29. Maung ZT, Reid MM, Matheson E, et al. Corticosteroid resistance is increased in lymphoblasts from adults compared with children: preliminary results of in vitro drug sensitivity study in adults with acute lymphoblastic leukaemia. *Br J Haematol* 1995;**91**:93–100.
30. Styczynski J, Pieters R, Huismans DR, et al. In vitro drug resistance profiles of adult versus childhood acute lymphoblastic leukaemia. *Br J Haematol* 2000;**110**:813–8.
31. Hoelzer D. Acute lymphoblastic leukemia: progress in children, less in adults. *N Engl J Med* 1993;**329**:1343–44.
32. Fiere D, Schaison G, Bancillon A, Sebban C. What is the best treatment for patients between 15 years and 20 years with, Comparative results of two protocols, one for acute lymphoblastic leukemia adults, one for children. *Blood* 1990;**76**(suppl 1):270a.
33. Stock W, La M, Sanford B, et al. What determines the outcomes for adolescents and young adults with acute lymphoblastic leukaemia treated on cooperative group protocols? A comparison of Children's Cancer Group and Cancer and Leukemia Group B studies. *Blood* 2008;**112**:1646-54.
34. Testi AM, Valsecchi MG. Difference in outcome of adolescents with acute lymphobastic leukaemia enrolled in pediatric (AIEOP) and adult (GIMEMA) protocols. *Blood* 2004; **104**(suppl):1954a.
35. de Bont JM, Holt B, Dekker AW, et al. Significant difference in outcome for adolescents with acute lymphoblastic leukemia treated on pediatric versus adult protocols in the Netherlands. *Leukemia* 2004; **18**(12):2032–5.
36. Hallbook H, Gustafsson G, Smedmyr B, et al. Treatment outcome in young adults and children > 10 years of age with acute lymphoblastic leukemia in Sweden: a comparison between a pediatric protocol and an adult protocol *Cancer* 2006;**107**(7):1551–61.
37. Ramanujachar R, Richards S, Hann I et al. Adolescents with acute lymphoblastic leukaemia: outcome on UK national paediatric (ALL97) and adult (UKALLXII/E2993) trials. *Pediatr Blood Cancer* 2007;**48**(3):254–61.
38. Leikin SL, Brubaker C, Hartmann JR, et al. Varying prednisone dosage in remission induction of previously untreated childhood leukemia. *Cancer* 1968;**21**:346–51.
39. Schwartz CL, Thompson EB, Gelber RD, et al. Improved response with higher corticosteroid dose in children with acute lymphoblastic leukemia. *J Clin Oncol* 2001;**19**:1040–6.
40. Silverman LB, Gelber RD, Dalton VK, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. *Blood* 2001;**97**:1211–8.
41. Amylon MD, Shuster J, Pullen J, et al. Intensive high-dose asparaginase consolidation improves survival for pediatric patients with T cell acute lymphoblastic leukemia and advanced stage lymphoblastic lymphoma: a Pediatric Oncology Group study. *Leukemia* 1999, **13**:335–42.
42. Riehm H, Gadner H, Henze G, et al. Results and significance of six randomized trials in four consecutive ALL-BFM studies. *Hamatol Bluttransfus* 1990;**33**:439–50.
43. Tubergen DG, Gilchrist GS, O'Brien RT, et al. Improved outcome with delayed intensification for children with acute lymphoblastic leukemia and intermediate presenting features: a Childrens Cancer Group phase III trial. *J Clin Oncol* 1993;**11**:527–37.
44. Nachman JB, Sather HN, Sensel MG, et al. Augmented post-induction therapy for children with high-risk acute lymphoblastic leukemia and a slow response to initial therapy. *N Engl J Med* 1998;**338**:1663–71.
45. Ramanujachar R, Richards S, Hann I, Webb D. Adolescents with acute lymphoblastic leukaemia: emerging from the shadow of paediatric and adult treatment protocols. *Pediatr Blood Cancer* 2006; **47**(6):748–56.
46. Ribera JM, Oriol A, Sanz MA et al. Comparison of the results of the treatment of adolescents and young adults with standard-risk acute lymphoblastic leukemia with the Programa Español de Tratamiento en Hematología pediatric-based protocol ALL-96. J Clin Oncol. 2008 Apr 10;26(11):1843-9.
47. Storring JM, Minden MD, Kao S et al. Treatment of adults with BCR-ABL negative acute lymphoblastic leukaemia with a modified paediatric regimen. Br J Haematol. 2009 Jun;146(1):76-85.
48. Huguet F, Leguay T, Raffoux E et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. J Clin Oncol. 2009 Feb 20;27(6):911-8. Erratum in: J Clin Oncol. 2009 May 20;27(15):2574. Dosage error in article text.

Ph+Acute Lymphoblastic Leukemia : Use of Tyrosine Kinase Inhibitors

Stephen P. Hunger

Abstract

Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL) has historically been one of the most difficult to treat subsets of childhood ALL. Based on knowledge of the molecular genetics and biology of *BCR-ABL1* fusion produced by the Ph+ in chronic myelogenous leukemia (CML) and Ph+ ALL, first (imatinib) and second (dasatinib and nilotinib) generation tyrosine kinase inhibitors (TKI) that target BCR-ABL1 were developed. These TKIs have revolutionized treatment of CML, and recent studies show that addition of imatinib to intensive chemotherapy leads to dramatic improvements in outcome of pediatric Ph+ ALL. These studies call for a reassessment of the routine use of stem cell transplantation (SCT) for all children with Ph+ ALL. The second generation TKIs have theoretical advantages over imatinib, but have not yet been used extensively in Ph+ ALL. In coming years, studies will define the optimal use of chemotherapy, SCT, and TKI in Ph+ ALL. New agents are being developed to circumvent resistance to first and second generation TKIs in Ph+ ALL and will likely be integrated into future treatment regimens for Ph+ ALL.

Identification of the Philadelphia chromosome and *BCR-ABL1* fusion

The Philadelphia chromosome (Ph) was first recognized as a small chromosome present in two patients with CML by Nowell and Hungerford in 1960, and then shown by Janet Rowley in 1973 to be the reciprocal translocation t(9;22)(q34;q11.2).(1,2) In the early 1980s, molecular investigations revealed that the chromosome 9 gene involved in this translocation was *ABL1*, (3) the human homologue of the Abelson murine leukemia virus, and that chromosome 22 genomic breakpoints were clustered within a region of 5.8 kilobases (kb) in what was subsequently called the Breakpoint Cluster Region gene (*BCR*).(4) Subsequent studies showed that the t(9;22) created chimeric *BCR-ABL1* transcripts that encoded for a fusion protein of 210 kD that had tyrosine kinase activity.(5) Transgenic mice that expressed BCR-ABL1 were generated and shown to have a myeloproliferative disorder similar to human CML.(6, 7) Critically, parallel studies established that the transforming potential of BCR-ABL1 was entirely dependent on an intact kinase domain.(8)

Development of imatinib and early testing in CML

Taken together, these observations suggested that agents that inhibited the tyrosine kinase activity of BCR-ABL1 might have therapeutic potential for CML. Brian Druker teamed with scientists at Ciba-Geigy (now Novartis) who were screening compounds to identify TKI. This collaboration eventually led to identification of STI571 (imatinib mesylate), which was found to be a potent and specific inhibitor of BCR-ABL1 that could kill CML cells in vitro.(9) A phase I dose escalation study of imatinib in patients with CML refractory to other therapies was begun in 1998.(10) Toxicity was mild in comparison to standard cytotoxic drugs and a maximally tolerated dose (MTD) was not identified when doses of up to 1000 mg/day were tested. The results of this trial were remarkable, with 98% (53/54) of patients with CML in chronic phase (CP) that were resistant/intolerant to interferon attaining a complete hematological response (CHR) when treated with at least 300 mg/day imatinib and 60% had a decrease of Ph+ metaphases to less than 35%.(10) This and other studies led to FDA approval of imatinib for the treatment of CML in 2000, and randomized trials showed imatinib to be the best available first line therapy for patients with CML-CP.(11)

Second generation Abl kinase inhibitors

A number of subsequent studies have shown that patients with CML can develop resistance to imatinib mediated by over-expression of BCR-ABL, or, more commonly, by point mutations in the Abl kinase domain that interfere with imatinib binding.(12) Two second generation Abl class TKIs have been developed to circumvent resistance—dasatinib and nilotinib. Similar to imatinib, nilotinib (formerly termed AMN107) binds only to the Abl class, KIT, and platelet derived growth factor receptor (PDGFR) TKs, but is 10- to 30-fold more potent than imatinib against BCR-ABL1 mutants resistant to imatinib, with the prominent except of the T315I mutation.(13) One of the most closely related kinases to Abl is Src, but imatinib and nilotinib do not inhibit Src kinase activity. Dasatinib, which was originally developed as a SRC kinase inhibitor, was found to be a potent inhibitor of BCR-ABL1 kinase activity (325 times more potent than imatinib in vitro), and active against most imatinib-resistant BCR-ABL1 mutants, again with the exception of T315I.(14) Both dasatinib and nilotinib are now FDA-approved for the treatment of patients with CML who are resistant to, or intolerant of, imatinib, and trials comparing these agents to imatinib are underway in CML.

Treatment of Ph+ ALL in the pre-imatinib era

In addition to its involvement in CML, the Ph+ also occurs in patients with acute lymphoblastic leukemia (ALL), although the genomic breakpoints are typically different leading to production of a 190 kD fusion protein in most cases. The Ph+ is present in about 3% of children with (ALL), about 90% of whom have the "ALL type" breakpoints that produce p190 BCR-ABL1.(15,16) The incidence of Ph+ ALL begins to increase in adolescence and the overall incidence in adults is 15-25% with rates increasing with age.(17)

Historically, Ph+ ALL has been one of the worst prognostic groups in pediatric ALL. In the largest study published to date, 326 children and adolescents less than 20 years old with Ph+ ALL diagnosed between 1986 and 1996 had a 7-year event-free survival (EFS) rate of 25% and overall survival (OS) rate of 36%.(15) In that study, matched related, but not unrelated donor stem cell transplantation (SCT) produced better outcomes than chemotherapy alone. A subsequent retrospective review of over 600 Ph+ ALL patients treated by fourteen pediatric cooperative groups from 1995-2005 showed modest improvements in outcome with 7-year EFS of 31% and OS of 44%.(18) This study included only patients who did not receive any TKI therapy, and thus serves as a baseline for future studies. SCT, using either matched related or unrelated donors, was a superior treatment strategy to chemotherapy, but results were still poor even with SCT.

Imatinib in Ph+ ALL

As imatinib was developed, a variety of studies showed that it was also effective in Ph+ ALL, but responses of patients with advanced disease to single agent therapy were typically very short-lived. Promising early results have been seen when imatinib was combined with chemotherapy in adults with Ph+ ALL, but the treatment strategies pursued typically focused on the use of SCT for consolidation therapy.(19, 20) The Children's Oncology Group (COG) AALL0031 trial (2002-2006) incorporated imatinib, starting after completion of induction therapy, into a very intensive chemotherapy regimen in a stepwise fashion, with SCT reserved, per study criteria, for those patients with a matched related donor.(21) Patients in the last cohort of AALL0031 (#5) received continuous treatment with imatinib 340 mg/m^2/day from the start of Consolidation, with the drug administered on a two week on/two week off schedule for the last year of maintenance therapy. The regimen was well tolerated, and there were no significant increased toxicities due to imatinib. Patients treated in cohort 5 had a 3-year EFS of 80%, which was more than double the EFS rate (35±4%; $p < 0.0001$) of historical controls treated in the pre-imatinib era. There was no advantage for SCT with 3-year EFS similar for patients in Cohort 5 treated with chemotherapy plus imatinib, related donor SCT, or off protocol therapy unrelated donor SCT. While these results are based on relatively small patient numbers, they have been stable with longer follow-up, and suggest that addition of imatinib to intensive chemotherapy can dramatically improve the outcome of children with Ph+ ALL, and thus call

for a reassessment of routine use of SCT in this disease. However, there were several potential disadvantages to the AALL0031 treatment strategy. First, imatinib treatment was not started until induction therapy was concluded. Consistent with historical data, (15, 18) about 10% of patients failed to enter remission after 4 weeks of chemotherapy. Second, the chemotherapy regimen administered intensive treatment for a prolonged time with high cumulative doses of many agents. It is not clear whether or not the intensive chemotherapy contributed to the observed improvements in outcome, or whether similar outcomes could be obtained with more standard chemotherapy regimens plus a TKI.

In parallel to COG AALL0031, the major European pediatric cooperative groups have conducted the EsPhALL study for children with Ph+ ALL. This study took a different approach and originally randomized low risk Ph+ ALL patients to receive chemotherapy +/- imatinib, with higher risk patients non-randomly assigned to the + imatinib arm. By design, the rates of SCT are much higher on the EsPhALL study than in COG AALL0031 and dose intensity of imatinib is lower. The EsPhALL trial has recently been amended to use imatinib in all patients with earlier, and more intensive use of this agent.

Unanswered questions and future directions

There are several important unanswered questions in pediatric Ph+ ALL, including: (1) What is the optimal TKI to combine with chemotherapy?; (2) How intensive a chemotherapy backbone is needed?; and (3) What is the role of SCT in Ph+ ALL?

The COG is currently conducting AALL0622 as a successor to AALL0031. The AALL0622 chemotherapy backbone is identical to that used in AALL0031 with minor exceptions. Several observations led the COG to conclude that that optimizing TKI therapy was the best way to improve outcomes in Ph+ ALL. These included the very promising results of AALL0031 and results of a GMALL study in elderly adults with Ph+ ALL.(22) In that study, adults older than 55 years of age received a 5-day chemotherapy prophase (dexamethasone 10 mg/m^2/day x 5 days, cyclophosphamide 200 mg/m^2 x 3, and one dose of intrathecal methotrexate) and then were randomized to receive a 4-week cycle of imatinib (600 mg/day) or a multiagent chemotherapy regimen. Following this, patients received chemotherapy + imatinib. The complete remission (CR) rate was much higher on the imatinib monotherapy arm (96% vs. 50%, p=0.0001) The COG also felt that available data suggested that dasatinib might be a more effective agent than imatinib for treatment of Ph+ ALL. In particular, in murine models signalling through SRC family kinases HCK, LYN, and FGR is required for development of Ph+ ALL, but not CML.(23) As noted above, dasatinib is a dual SRC/ABL TKI that is 325-times more potent than imatinib against BCR-ABL1 in vitro, and has activity against most imatinib-resistant BCR-ABL1 mutants. Finally, unlike imatinib, dasatinib crosses the blood-brain barrier and is effective treatment for central nervous system leukaemia in patients with Ph+ ALL.(24) Based on these data, AALL0622 uses dasatinib rather than imatinib, and also starts dasatinib therapy at day 15 of Induction. This timepoint was selected as it was felt to be the earliest time that was feasible for a large study that involves more than 100 centers.

AALL0622 was designed before the results of AALL0031 were available and includes options for matched related donor SCT for all patients, and matched unrelated donor SCT for patients with a poor early response to therapy, defined as minimal residual disease levels (measured by flow cytometry) of >1% at end induction or >0.01% at end of 2 months of consolidation therapy.

Future studies in Ph+ ALL will continue to focus on defining the optimal chemotherapy backbone, the optimal TKI, and the role of SCT in CR1. It will also be critical to develop new strategies for treatment of patients that have BCR-ABL1 point mutations that are resistant to the currently available 1st and 2nd generation TKIs. The most resistant point mutation in CML is T315I, which also occurs in Ph+ ALL. A number of agents, including Aurora kinase inhibitors, have been developed that can inhibit this and other highly resistant BCR-ABL1 mutations.(25, 26) One can anticipate that such agents might be combined with chemotherapy and a TKI to treat Ph+ ALL in the future.

Summary

The treatment of Ph+ leukemias is a paradigm for how molecularly targeted therapies can improve outcomes in human cancer. Because Ph+ ALL is a more "virulent" disease than CML (with more accumulated genetic lesions), TKI monotherapy is ineffective. However, combination regimens of chemotherapy + TKIs hold great promise for treatment of this disease. There are major questions remaining about how Ph+ pediatric ALL should be treated optimally. We can only hope that the next decade will be as productive as the past one has been in improving outcome for this once very recalcitrant subtype of leukaemia.

References

1. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst. 1960 Jul;25:85-109.
2. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973 Jun 1;243(5405):290-3.
3. de Klein A, van Kessel AG, Grosveld G, Bartram CR, Hagemeijer A, Bootsma D, et al. A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. Nature. 1982 Dec 23;300(5894):765-7.
4. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell. 1984 Jan;36(1):93-9.
5. Davis RL, Konopka JB, Witte ON. Activation of the c-abl oncogene by viral transduction or chromosomal translocation generates altered c-abl proteins with similar in vitro kinase properties. Mol Cell Biol. 1985 Jan;5(1):204-13.
6. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science. 1990 Feb 16;247(4944):824-30.
7. Heisterkamp N, Jenster G, ten Hoeve J, Zovich D, Pattengale PK, Groffen J. Acute leukaemia in bcr/abl transgenic mice. Nature. 1990 Mar 15;344(6263):251-3.
8. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science. 1990 Mar 2; 247(4946):1079-82.
9. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996 May;2(5):561-6.
10. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, et al. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med. 2001 Apr 5;344(14):1038-42.
11. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med. 2003 Mar 13;348(11):994-1004.
12. Shah NP, Sawyers CL. Mechanisms of resistance to STI571 in Philadelphia chromosome-associated leukemias. Oncogene. 2003 Oct 20;22(47):7389-95.
13. Weisberg E, Manley PW, Breitenstein W, Bruggen J, Cowan-Jacob SW, Ray A, et al. Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell. 2005 Feb;7(2):129-41.
14. Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, et al. Discovery of N-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl)- piperazin-1-yl)-2-methylpyrimidin-4- ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. J Med Chem. 2004 Dec 30;47(27):6658-61.
15. Arico M, Valsecchi MG, Camitta B, Schrappe M, Chessells J, Baruchel A, et al. Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med. 2000 Apr 6;342(14):998-1006.
16. Suryanarayan K, Hunger SP, Kohler S, Carroll AJ, Crist W, Link MP, et al. Consistent involvement of the bcr gene by 9;22 breakpoints in pediatric acute leukemias. Blood. 1991 Jan 15;77(2):324-30.
17. Advani AS, Hunger SP, Burnett AK. Acute leukemia in adolescents and young adults. Semin Oncol. 2009 Jun;36(3):213-26.
18. Aricò M, Schrappe M, Hunger SP, Carroll WL, Conter V, Galimberti S, et al. Clinical outcome of 610 children with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia treated between 1995 and 2005. Journal of Clinical Oncology, In Press..
19. Yanada M, Takeuchi J, Sugiura I, Akiyama H, Usui N, Yagasaki F, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. J Clin Oncol. 2006 Jan 20;24(3):460-6.
20. de Labarthe A, Rousselot P, Huguet-Rigal F, Delabesse E, Witz F, Maury S, et al. Imatinib combined with induction or consolidation chemotherapy in patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: results of the GRAAPH-2003 study. Blood. 2007 Feb 15;109(4):1408-13.
21. Schultz KR, Bowman WP, Aledo A, Slayton WB, Sather H, Devidas M, et al. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute

lymphoblastic leukemia: a children's oncology group study. J Clin Oncol. 2009 Nov 1;27(31):5175-81.

22. Ottmann OG, Wassmann B, Pfeifer H, Giagounidis A, Stelljes M, Duhrsen U, et al. Imatinib compared with chemotherapy as front-line treatment of elderly patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL). Cancer. 2007 May 15;109(10):2068-76.

23. Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, et al. Requirement of Src kinases Lyn, Hck and Fgr for BCR-ABL1-induced B-lymphoblastic leukemia but not chronic myeloid leukemia. Nat Genet. 2004 May;36(5):453-61.

24. Porkka K, Koskenvesa P, Lundan T, Rimpilainen J, Mustjoki S, Smykla R, et al. Dasatinib crosses the blood-brain barrier and is an efficient therapy for central nervous system Philadelphia chromosome-positive leukemia. Blood. 2008 Aug 15;112(4):1005-12.

25. O'Hare T, Eide CA, Tyner JW, Corbin AS, Wong MJ, Buchanan S, et al. SGX393 inhibits the CML mutant Bcr-AblT315I and preempts in vitro resistance when combined with nilotinib or dasatinib. Proc Natl Acad Sci U S A. 2008 Apr 8;105(14):5507-12.

26. Fei F, Stoddart S, Groffen J, Heisterkamp N. Activity of the Aurora kinase inhibitor VX-680 against Bcr/Abl-positive acute lymphoblastic leukemias. Mol Cancer Ther. May;9(5):1318-27.

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The Acute Lymphoblastic Leukemias of Down Syndrome (DS-ALL)

Shai Izraeli

Abstract

Children with Down Syndrome have a markedly increased risk for acute lymphoblastic leukemia (DS-ALL). These leukemias are exclusively of the B cell precursor phenotype and occur in a similar age to "common" sporadic ALLs with the strike absence of infant leukemia. Recent studies reveal that DS-ALLs are heterogeneous and differ from sporadic ALLs. Only about a fifth of DS-ALLs carry the common cytogenetic aberrations typical to sporadic ALL. Genomic rearrangements leading to the expression of a cytokine receptor, CRLF2, are detected in 60% of DS-ALL in comparison with 10% of sporadic ALLs. These abnormalities are often associated with acquired mutations in the JAK-STAT pathway. In general, the prognosis of DS-ALL is inferior to sporadic ALL mainly because of increased treatment toxicity. However recent data challenge this view and suggest that the inferior outcome may be mainly related to the genetic properties of the leukemic cells and that excessive chemotherapy dose reductions may not be appropriate for these patients. The common activation of the CRLF2-JAK-STAT signaling pathway in DS-ALLs suggests a future for targeted therapy with JAK inhibitors for DS-ALLs.

Children with Down Syndrome (DS) have a markedly enhanced incidence of myeloid (ML-DS) and lymphoid (DS-ALL) leukemias. The risk of DS-ALL has been estimated to be 10-20 times higher than sporadic ALL[1-2]. In most published multi-institutional ALL protocols DS-ALL comprises about 1-3% of total patients [3-6]. The higher risk of leukemias in DS is striking in light of the *reduced* risk of most solid tumors [1, 7]. This suggests a leukemogenic role of constitutional trisomy 21.

The ML-DS is a defined entity, unique to DS, with a clear clinical presentation and course, excellent response to chemotherapy and a relatively well deciphered molecular pathogenesis [8-9]. In contrast, at first glance DS-ALL resembles B cell precursor ALL (BCP-ALL) in children without DS. They have a similar clinical appearance to the "common" B cell precursor (BCP) ALLs seen in children without DS, with the notable absence of infant leukemias[10]. The peak age is about 5 years, the immunophenotype is typical for B-cell precursor (BCP) ALL, namely positive for CD10, CD19, and CD79a and they usually classified into the standard National Cancer Institute risk group. Yet recent studies have demonstrated that this resembles is misleading. Here this recent research and the challenges in clinical management of ALLs in DS are reviewed.

Pathogenesis

The excess of ALLs in DS raises several general questions:

a) Are these leukemias unique to DS (like the ML-DS) or do children with DS have a general increased risk for childhood "common" B cell precursor ALL?

b) What is the nature of the acquired somatic genetic events that cooperate with constitutional trisomy 21 in the evolution to ALL? Since most children with DS do not develop leukemia, such progression events are necessary for leukemogenesis. Almost all the ML-DS have an acquired mutation in the megakaryocytic transcription factor GATA1 [11-12]. Does a similar cooperative genetic event, unique to DS, exist in DS-ALL?

c) What is the role of trisomy 21? Which are the genes on trisomy 21 that confers increased risk for ALL?

While ML-DS is a unique disease (that has now a special WHO classification code) recent studies demonstrate that DS-ALL is a

heterogeneous disease suggesting complex pathogenesis. This is clearly evident in the gene expression patterns. Unlike the usual genetic subtypes of childhood ALL that are clearly clustered in distinct subgroups by gene expression profiling, DS-ALLs do not fall into one clear diagnostic cluster. Thus the term "DS-ALL" may be a misnomer – there are different ALLs in DS.

Although the immunophenotype of DS-ALL is of a typical childhood BCP-ALL, there is a significantly lower prevalence of the common genetic subtypes of B cell precursor ALL (BCR/ABL, TEL/AML1 and Hyeprdiploid ALL (HHD)) in DS-ALL [6, 13-19]. This was confirmed in two recent large studies – a retrospective database of the iBFM study group consisting of 215 DS-ALLs compiled from several cytogenetics laboratories[20] and a prospective systematic diagnostic genetic testing of childhood ALL in Children's Oncology Group (COG) trials during the last decade[6]. Both suggest that only up to one fifth of DS-ALLs carry the two most frequent genetic anomalies, HHD and TEL/AML1, characterizing about 60% of sporadic childhood BCP-ALL. However, if one take into account the 20 fold increased risk of ALL in DS[1], then there may be an *absolute increase* in the incidence of these common subtypes of childhood leukemias in DS.

It emerges that the majority of the DS-ALLs differ from the sporadic ALLs containing excess chromosome 21. The most common cytogenetic abnormality in DS-ALL is an extra chromosome X, observed in close to half the patients [20]. Additional copy of chromosome X is usually present in HHD sporadic ALL however the combination of trisomy 21 and extra chromosome X as a *single cytogenetic abnormality* seems unique to DS-ALL, and suggest a, yet unknown, collaborating event between gene (s) on chromosome 21 and X.

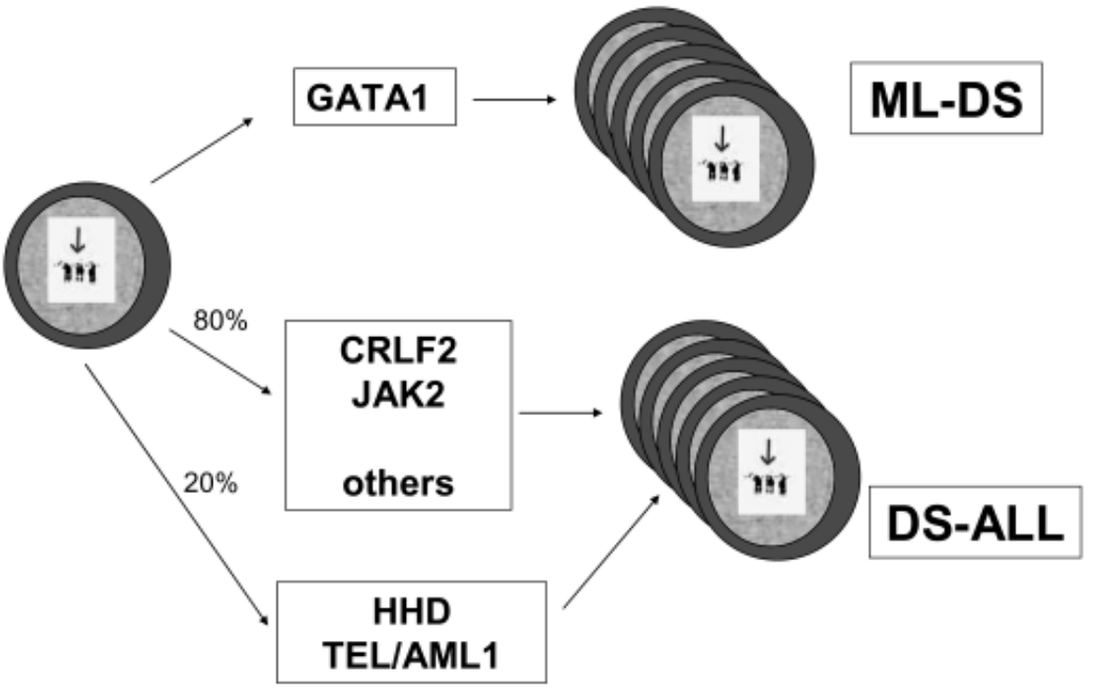


Figure 1.

Somatic genetic events cooperating with constitutional trisomy 21 in initiation of leukemias. *The myeloid leukemias of DS (ML-DS) are universally characterized by an acquired mutation in GATA1. The lymphoid leukemias (DS-ALL) are more heterogeneous. About 20% of DS-ALLs carry similar aberrations of sporadic ALLs, namely TEL/AML1 translocation or hyperdiploidy (HHD). Most of DS-ALLs have cooperating events that are relatively unique to DS. About 60% have an aberrant expression of CRL2 often associated with JAK2 mutations. It is likely that additional somatic mutations are necessary for progression from the pre-luekemic phase to full blown leukemias.*

Such a collaborating event has recently been discovered. Genomic aberrations causing the expression of cytokine receptor CRLF2 are present in about 60% of DS-ALLs[21-23] but only in up to 10% of sporadic childhood ALL[23-25]. CRLF2 encodes one chain of the receptor to TSLP, a cytokine involved in allergic and inflammatory disorders. It signals into the cells via the JAK-STAT pathway. The importance of the activation of this pathway to survival of the leukemia cells is underscored by the frequent occurrence of activating mutations in the kinases JAK2 or JAK1 or in the CRLF2 receptor itself[21, 26]. The presence of activation of the JAK-STAT pathway in the majority of DS-ALLs, expressing CRLF2, suggest that these leukemias may be candidates for therapy with the novel JAK2 inhibitors that are being explored in early clinical trials for myeloproliferative neoplasms.

The role of the trisomy 21 has remained a mystery. There is high interest in the pathogenesis of DS-ALL also because trisomy 21 (or sometimes tetrasomy 21) is the most common *acquired somatic* chromosomal abnormalities in sporadic ALL [27]. It is mostly found in HHD-ALL a subtype of ALL characterized by more than 50 chromosomes, always involving chromosome 21. Hence it is tempting to speculate that constitutional and somatic trisomy 21 may facilitate leukemogenesis in a similar fashion and therefore the study of DS ALL may have direct implications for sporadic childhood ALL. Indeed gene expression analysis demonstrates that level of expression of chromosome 21 genes is similar in HHD and DS ALLs [21]

Yet, as have been recently demonstrated, HHD and DS ALLs differ significantly for example by the abnormal expression of CRLF2 and the associated mutations in JAK2. Importantly there are fundamental differences between *constitutional* and *somatic* trisomies that could explain the uniqueness of DS leukemias. The former exists in all body cells from the time of conception, whereas the latter is acquired and exists only in the transformed cells. Thus constitutional trisomy can predispose to cancer in a variety of ways. It may exert a *direct* activity in a *cell autonomous* manner enhancing the risk of transformation or affecting the differentiation of (fetal) B cell progenitors. Alternatively, the trisomy could promote leukemia because of aberrant effects on immediate *micro-environment*, for example on the bone marrow's or fetal liver's stroma cells that regulate proliferation and differentiation of hematopoietic stem cells. More complex may be the influence of the trisomy on the macro-environment. For example, viral infections and the immunological response have been suggested to have a role in the pathogenesis of childhood common ALL [28-29] The markedly increased risk of ALL in DS could also be caused by the immunodeficiency, altered immunological environment and the increased infection rate that characterize DS.

Why aberrant expression of CRLF2 is so much common in DS-ALL compared with sporadic ALL is unknown. Perhaps CRLF2 expressing cells are selected by increased production of TSLP in the bone marrow of children with DS, but this has not been shown yet. Anther possibility is that a prolonged arrest in early B cell developmental stages in which the V(D)J recombination machinery is active might explain the chromosomal aberrations involving CRLF2 or other translocations to the IgH locus that are more frequent in DS-ALL, such as the t(8;14)(q11;q32)[20]. Consistent with this hypothesis are the aberrant expression of DNA damage genes in DS-ALL suggesting the presence of lymphocytic specific genomic instability[21].

Clinical course and therapy

Unlike ML-DS that is uniquely sensitive to chemotherapy, in particular to ARA-C, the prognosis of DS-ALL is less favorable in most of the clinical trials[15-16, 18-19, 30-34]. Marked toxicity manifested by increased mucositis, infections and death during intensive periods of chemotherapy is observed. DS patients may be especially sensitive to the toxic effects of Methotrexate, a drug that is not used in AML, due to the excess activity of the folate transporter coded by a gene on chromosome 21 [35]. However, severe toxicity is also observed to anthracyclines and to the marked immunosuppressive effect of ALL therapy.

Importantly, marked reduction of chemotherapy may be a mistake in DS-ALL. A Children's Cancer Group study demonstrated a surprisingly good survival in children with DS-ALL treated by

risk adjusted intensive chemotherapy protocols Event-free (56% vs. 74%; P < .001) and disease-free (55% vs. 73%; P < .001) survival at 10 years was significantly lower in the standard-risk DS-ALL population compared with ALL in non DS, but not in high-risk DS-ALL population (event-free survival, 62% vs. 59%; P = .9; disease-free survival, 64% vs. 59%; P = .9), and these differences persisted regardless of treatment era (early era [1983-1989] vs. recent era [1989-1995])[31]. These observations have been recently confirmed by analysis of COG trials. It demonstrated that the major cause of the poorer outcome of DS-ALL is the lower prevalence of the good prognostic sentinel cytogentic lesions, namely TEL/AML1 fusion and trisomies of chromosomes four and ten[6].

These results suggest that intensification of therapy for patients with DS-ALL is needed to maintain outcome comparable with those of ALL in non DS patients. Similarly a recent survey of 8 children with DS-ALL who underwent bone marrow transplantation reported that relapse and not treatment related toxicity were the major causes for treatment failure. Indeed the only surviving patients were those that were treated by myeloablative chemotherapy [32].

Thus the clinician faced with a patient with DS and ALL has difficult choices. Intensive chemotherapy is likely to cause life endangering toxicity but may also be required for cure, especially if the leukemia lacks the ETV6-RUNX1 translocation or hyperdiploidy. There may be a light in the end of this tricky maze. The activation of the CRLF2-JAK-STAT signaling pathway in the majority of DS-ALLs suggests a therapeutic potential for JAK inhibitors. If confirmed in clinical trials, this therapy will target the unique biological properties of ALLs in children with DS.

References

1. Hasle H. Pattern of malignant disorders in individuals with Down's syndrome. Lancet Oncol 2001;2:429-36.
2. James R, Lightfoot T, Simpson J, Moorman AV, Roman E, Kinsey S. Acute leukemia in children with Down's syndrome: the importance of population based study. Haematologica 2008;93:1262-3.
3. Ravindranath Y. Down syndrome and leukemia: new insights into the epidemiology, pathogenesis, and treatment. Pediatr Blood Cancer 2005;44:1-7.
4. Ross JA, Spector LG, Robison LL, Olshan AF. Epidemiology of leukemia in children with Down syndrome. Pediatr Blood Cancer 2005;44:8-12.
5. Zeller B, Gustafsson G, Forestier E, et al. Acute leukaemia in children with Down syndrome: a population-based Nordic study. Br J Haematol 2005;128:797-804.
6. Maloney KW, Carroll WL, Carroll AJ, et al. Down syndrome childhood acute lymphoblastic leukemia has a unique spectrum of sentinel cytogenetic lesions that influences treatment outcome: a report from the Children's Oncology Group. Blood 2010.
7. Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet 2000;355:165-9.
8. Malinge S, Izraeli S, Crispino JD. Insights into the manifestations, outcomes and mechanisms of leukemogenesis in Down syndrome. Blood 2009.
9. Izraeli S, Rainis L, Hertzberg L, Smooha G, Birger Y. Trisomy of chromosome 21 in leukemogenesis. Blood Cells, Molecules, and Diseases 2007;39:156-9.
10. Malinge S, Izraeli S, Crispino JD. Insights into the manifestations, outcomes, and mechanisms of leukemogenesis in Down syndrome. Blood 2009;113:2619-28.
11. Rainis L, Bercovich D, Strehl S, et al. Mutations in exon 2 of GATA1 are early events in megakaryocytic malignancies associated with trisomy 21. Blood 2003;102:981-6.
12. Wechsler J, Greene M, McDevitt MA, et al. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet 2002;32:148-52.
13. Pui CH, Raimondi SC, Borowitz MJ, et al. Immunophenotypes and karyotypes of leukemic cells in children with Down syndrome and acute lymphoblastic leukemia. J Clin Oncol 1993;11:1361-7.
14. Savasan S, Taub JW, Ravindranath Y. Down syndrome and leukemia—an overview of cytogenetic and molecular events. Turk J Pediatr 1997;39:519-31.
15. Dordelmann M, Schrappe M, Reiter A, et al. Down's syndrome in childhood acute lymphoblastic leukemia: clinical characteristics and treatment outcome in four consecutive BFM trials. Berlin-Frankfurt-Munster Group. Leukemia 1998;12:645-51.
16. Chessells JM, Harrison G, Richards SM, et al. Down's syndrome and acute lymphoblastic leukaemia: clinical features and response to treatment. Arch Dis Child 2001;85:321-5.
17. Bassal M, La MK, Whitlock JA, et al. Lymphoblast biology and outcome among children with Down syndrome and ALL treated on CCG-1952. Pediatr Blood Cancer 2005;44:21-8.
18. Whitlock JA. Down syndrome and acute lymphoblastic leukaemia. Br J Haematol 2006;135:595-602.
19. Arico M, Ziino O, Valsecchi MG, et al. Acute lymphoblastic leukemia and Down syndrome: presenting features and treatment outcome in the experience of the Italian Association of Pediatric Hematology and Oncology (AIEOP). Cancer 2008;113:515-21.

20. Forestier E, Izraeli S, Beverloo B, et al. Cytogenetic features of acute lymphoblastic and myeloid leukemias in pediatric patients with Down syndrome: an iBFM-SG study. Blood 2008;111:1575-83.

21. Hertzberg L, Vendramini E, Ganmore I, et al. Down syndrome acute lymphoblastic leukemia, a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the International BFM Study Group. Blood 2010;115:1006-17.

22. Mullighan CG, Collins-Underwood JR, Phillips LA, et al. Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. Nat Genet 2009;41:1243-6.

23. Russell LJ, Capasso M, Vater I, et al. Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. Blood 2009;114:2688-98.

24. Cario G, Zimmermann M, Romey R, et al. Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. Blood 2010.

25. Harvey RC, Mullighan CG, Chen IM, et al. Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. Blood 2010.

26. Bercovich D, Ganmore I, Scott LM, et al. Mutations of JAK2 in acute lymphoblastic leukaemias associated with Down's syndrome. Lancet 2008;372:1484-92.

27. Heerema NA, Sather HN, Sensel MG, et al. Prognostic impact of trisomies of chromosomes 10, 17, and 5 among children with acute lymphoblastic leukemia and high hyperdiploidy (> 50 chromosomes). J Clin Oncol 2000;18:1876-87.

28. Einav U, Tabach Y, Getz G, et al. Gene expression analysis reveals a strong signature of an interferon-induced pathway in childhood lymphoblastic leukemia as well as in breast and ovarian cancer. Oncogene 2005;24:6367-75.

29. Greaves MF. Aetiology of acute leukaemia. Lancet 1997;349:344-9.

30. Watson MS, Carroll AJ, Shuster JJ, et al. Trisomy 21 in childhood acute lymphoblastic leukemia: a Pediatric Oncology Group study (8602). Blood 1993;82:3098-102.

31. Whitlock JA, Sather HN, Gaynon P, et al. Clinical characteristics and outcome of children with Down syndrome and acute lymphoblastic leukemia: a Children's Cancer Group study. Blood 2005;106:4043-9.

32. Meissner B, Borkhardt A, Dilloo D, et al. Relapse, not regimen-related toxicity, was the major cause of treatment failure in 11 children with Down syndrome undergoing haematopoietic stem cell transplantation for acute leukaemia. Bone Marrow Transplant 2007;40:945-9.

33. Bohnstedt C, Taskinen M, Zeller B, Bjorgvinsdottir H, Hafsteinsdottir S, Schmiegelow K. Poor treatment compliance in children with down syndrome and acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2009;31:79-80.

34. Shah N, Al-Ahmari A, Al-Yamani A, Dupuis L, Stephens D, Hitzler J. Outcome and toxicity of chemotherapy for acute lymphoblastic leukemia in children with Down syndrome. Pediatr Blood Cancer 2009;52:14-9.

35. Matherly LH, Taub JW. Methotrexate pharmacology and resistance in childhood acute lymphoblastic leukemia. Leuk Lymphoma 1996;21:359-68.

Genetic Alterations in High-Risk B-Progenitor Acute Lymphoblastic Leukemia

Charles G. Mullighan

Abstract

Recent studies profiling genetic alterations in B-progenitor acute lymphoblastic leukemia (B-ALL) at high resolution have identified multiple recurring submicroscopic genetic alterations targeting key cellular pathways in lymphoid cell growth, differentiation and tumor suppression. A key finding has been that genetic alterations disrupting normal lymphoid growth and differentiation and are associated with treatment outcome. Notably, genetic alterations targeting lymphoid development are present in over two-thirds of B-ALL cases, including deletions, translocations and sequence mutations of the transcriptional regulators *PAX5, IKZF1,* and *EBF1.* Deletion or mutation of the early lymphoid transcription factor gene *IKZF1* is hallmark of multiple subtypes of ALL with poor prognosis, including *BCR-ABL1* positive (Ph+) lymphoid leukemia and a novel subset of "BCR-ABL1-like" ALL cases that have a gene expression profile similar to that of Ph+ B-ALL, but lack expression of BCR-ABL1. In addition to deletion of *IKZF1*, these BCR-ABL1-like cases commonly harbor genetic mutations resulting in aberrant lymphoid cytokine receptor signaling, including activating mutations of Janus kinases and rearrangement of *CRLF2* (cytokine receptor-like factor 2). These findings demonstrate that multiple genetic alterations disrupting different cellular pathways are key events in the pathogenesis of high risk ALL, and suggest that novel therapies targeting aberrant cytokine receptor signaling may be of therapeutic benefit in high risk ALL cases.

Acute lymphoblastic leukemia (ALL) is the commonest childhood cancer (1,2), and despite impressive advances in the outcome of therapy with cure rates now exceeding 80% (3, 4), remains a leading cause of cancer-related death in children and young adults (5-7). Moreover, with increasing age through adolescence and adulthood, cure rates for ALL fall sharply, and the genetic and biologic determinants of treatment failure remain incompletely understood (8,9).

ALL has long been exceptionally well characterized at the cytogenetic level, and approximately three quarters of childhood ALL cases harbor recurring gross genetic alterations, including chromosomal aneuploidy (high hyperdiploidy and hypodiploidy), and rearrangements that dysregulate hematopoietic regulators, transcription factors, and tyrosine kinases. These include rearrangements resulting in (e.g. *ETV6-RUNX1*, *TCF3-PBX1*, *BCR-ABL1,* rearrangement of *MLL*, and rearrangements of T cell receptor genes to hematopoietic regulators and transcription factors in T-lineage ALL) (10,11). While alterations associated with favorable outcome (e.g. high hyperdiploidy and *ETV6-RUNX1*) are characteristic of childhood ALL, and the frequency of *BCR-ABL1* (Philadelphia chromosome positive, or Ph+) ALL rises with age (12), the differences in the frequencies of these recurring gross chromosomal rearrangements is insufficient to fully explain treatment failure in ALL, which occurs across the spectrum of cytogenetic subtypes, including cases that lack cytogenetic alterations. Consequently, there has been great interest in using genome-wide approaches to identify submicroscopic genetic alterations in ALL, and these studies have proven exceptionally fruitful in identifying new mutations that target key cellular pathways in B-progenitor and T-lineage ALL. Similar studies have also been informative in T-lineage ALL (13-16), however this review focuses primarily on B-progenitor ALL and recent studies that have identified genetic markers of treatment failure in this disease.

The spectrum of genetic alterations in ALL – insights from genome-wide profiling

Multiple studies have used array-based comparative genomic hybridization and single nucleotide polymorphism (SNP) microarrays to identify submicroscopic genetic alterations in childhood ALL. Fewer studies have examined young adult and older patients with ALL, and detailed profiling of these cohorts is required. In contrast to many solid tumors, which commonly harbor multiple gross DNA copy number alterations (17), ALL his characterized by a relatively low number of genetic alterations – approximately six to eight lesions per case (13, 18). However, recurring submicroscopic genetic alterarations targeting key cellular pathways and genes with key roles in leukemia development are a hallmark of ALL (13, 19, 20). These include mutations targeting transcriptional regulators of lymphoid development (e.g. *PAX5, IKZF1* and *EBF1*), cell cycle regulators and tumor suppressor genes (*CDKN2A, CDKN2B, RB1* and *PTEN*), lymphoid signaling genes (*CD200, BTLA* and *BLNK*) and drug response genes (e.g. the glucocorticoid receptor gene *NR3C1*). Genes regulating B lymphoid development are mutated in the majority of B-progenitor ALL cases, most commonly deletions, sequence mutations or translocations of *PAX5* (13, 19-21), deletion (and less commonly, sequence mutation) of *IKZF1* (IKAROS) and the IKAROS family members *IKZF2* (HELIOS) and *IKZF3* (AIOLOS) and deletion of *EBF1*. These mutations result in loss of function *in vitro* (13), and accelerate the onset of ALL in murine models of ALL (22-24). While the mutations most commonly involve only a single copy of the affected gene, multiple mutations involving this pathway are common in high-risk B-ALL, and a higher number of lesions in the pathway is associated with poor outcome (25), suggesting the degree of "block" in B cell differentiation induced by mutations in this pathway not only contributes to leukemogenesis, but also treatment responsiveness.

Genomic profiling of high-risk ALL – a central role of *IKZF1*

The frequency and nature of submicroscopic genetic alterations in ALL is strongly associated with disease subtype. *MLL*-rearranged ALL cases harbor fewer than one copy number alteration per case. This suggests that few cooperating structural genetic alterations are required to induce leukemia (13, 26). In contrast, *ETV6-RUNX1* and *BCR-ABL1* (Ph+) ALL cases harbor multiple distinct copy number alterations (13, 27). Deletion of *IKZF1* (IKAROS) is a hallmark of Ph+ lymphoid leukemia, including both childhood and adult *de novo* ALL cases (18, 28) and chronic myeloid leukemia (CML) at progression to lymphoid blast crisis (18, 28). Moreover, the presence of *IKZF1* alterations is associated with poor outcome in Ph+ ALL (29). IKAROS is a member of a family of zinc-finger transcription factors that has multiple, incompletely understood functions in lymphoid development and leukemogenesis, including transcriptional regulation and chromatin remodeling. Normal IKAROS function is required for the development of all lymphoid lineages (30-34, 35). Expression of aberrant IKAROS isoforms in ALL blasts is well recognized, notably that of one isoform, IK6. This isoform lacks the N-terminal zinc fingers of IKAROS and cannot bind DNA, but retains the C-terminal zinc fingers and can act in a dominant-negative fashion (36-38). However, SNP array profiling studies of both ALL and CML have shown that expression of these dominant-negative transcripts is determined by the presence of *IKZF1* deletions that involve the exons corresponding to those deleted in the aberrant *IKZF1* transcripts and IKZF1 protein (18,28).

Alterations in IKAROS function have previously been reported to have an important role in the pathogenesis of lymphoid tumors. Mice harboring a dominant-negative mutation in the *Ikzf1* gene develop aggressive T-lineage lymphoproliferative disease (39). Although the role of IKAROS in the pathogenesis of Ph+ ALL remains to be fully defined, existing data have shown that expression of IK6 impairs B lymphoid maturation (40, 41) and pre-B cell receptor signaling in Ph+ ALL cells (42). Moreover, deletion of *Ikzf1* accelerates leukemogenesis in a murine model of Ph+ ALL (24).

A role for IKAROS in the pathogenesis of ALL is also supported by recent data from genome-wide association studies in which an inherited SNP allele at the *IKZF1* locus was associated with the risk of childhood ALL, a finding that has

been identified in multiple studies and patient cohorts (43-45). The mechanistic basis of this finding remains unclear, although it is notable that *IKZF1* genotype was associated with the level of expressionof IKAROS (43), and the genes at the two other loci found to be associated with ALL risk in these studies, *ARID5B* and *CEBPE*, also encode transcriptional regulators and genes involved in lymphoid maturation (46,47), suggesting that germline variation at these loci directly influences the risk of ALL.

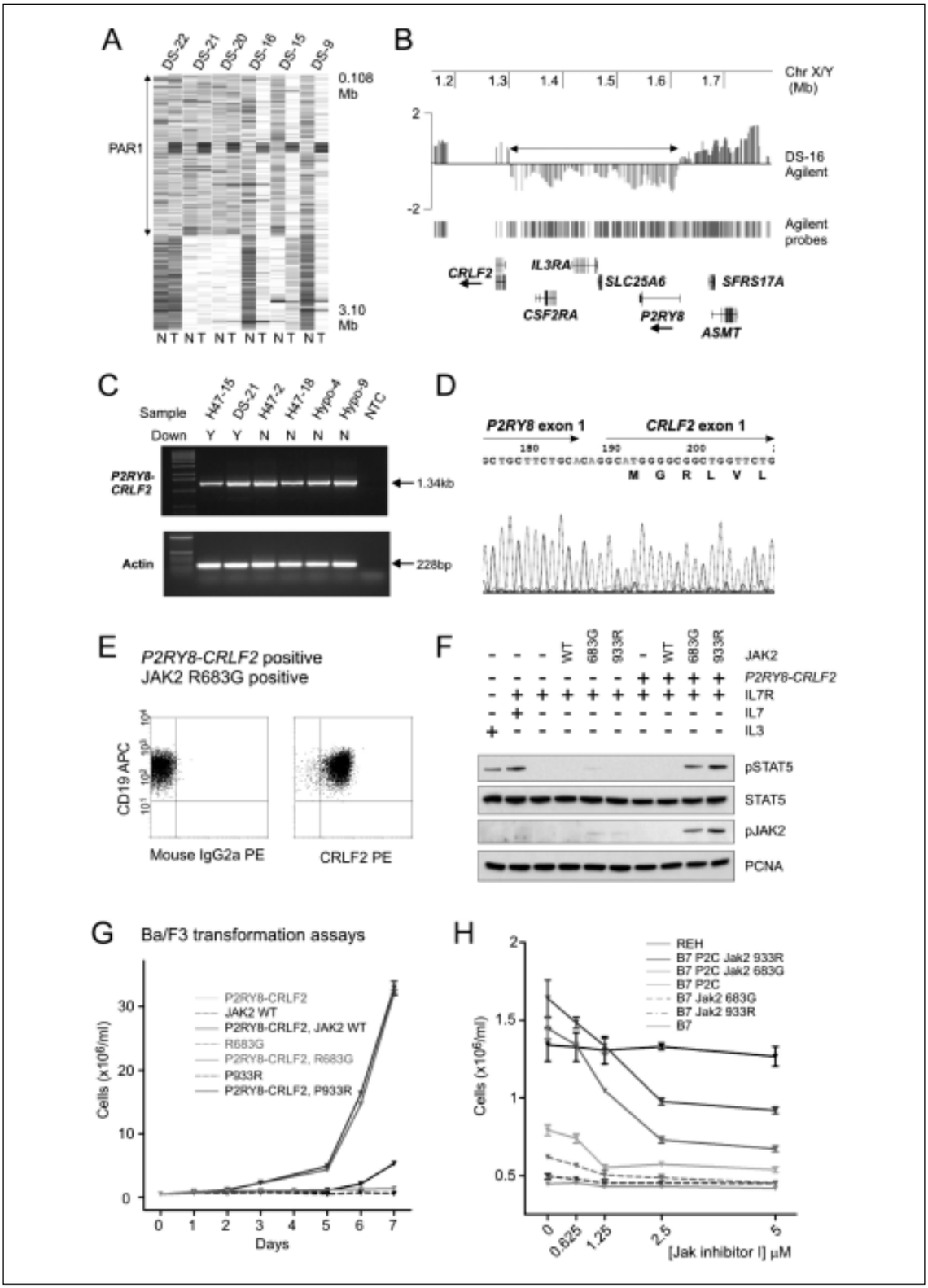
Alterations of *IKZF1* are also associated with poor outcome in Ph- ALL. A Children's Oncology Group study of over 200 cases of high-risk B-progenitor Ph-negative ALL identified *IKZF1* deletions and sequence mutations in approximately one third of cases. This study also found that *IKZF1* alteration was associated with a near tripling of the risk of treatment failure (25). This strong association between *IKZF1* and adverse outcome was confirmed in the Dutch DCOG-ALL9 cohort (48). In addition, profiling of serial ALL samples have identified substantial differences in the genetic alterations present at diagnosis and relapse. However, *IKZF1* alterations are almost always preserved from diagnosis to relapse, and may also be acquired as a new lesion at relapse (49-51). Together, these findings add weight to the data from Ph+ leukemia that alteration of *IKZF1* is a key determinant of leukemogenesis and response to therapy.

An additional notable observation is that the gene expression profile of poor outcome, *IKZF1*-altered B-progenitor ALL is strikingly similar to that of Ph+ ALL (25). A similar subtype of "BCR-ABL1-like" ALL enriched for genetic alterations targeting B lymphoid development has also been described by den Boer *et al* (52). The similarity of the gene expression profiles of *IKZF1*-deleted Ph+ and Ph- ALL suggests that perturbation of IKZF1 activity may directly influence the leukemic transcriptome and the degree of differentiation of ALL cells. Consistent with this, the gene expression profile of *IKZF1*-mutated Ph- ALL exhibits enrichment for hematopoietic stem cell genes and reduced expression of B cell signaling genes (25). In addition, *IKZF1* mutated, BCR-ABL1-like harbor mutations that result in activation of downstream signaling pathways similar to those activated by BCR-ABL1. A substantial proportion of BCR-ABL1-like ALL cases have genetic alterations that result in aberrant cytokine receptor signaling, notably activating Janus kinase (JAK) mutations and rearrangement of *CRLF2* (encoding the lymphoid cytokine receptor gene cytokine receptor like factor 2).

Genetic characterization of BCR-ABL1-like, Ph negative ALL

Detailed candidate gene resequencing in high risk ALL, including targets of DNA copy number alteration, dysregulated gene expression, and a subset of receptor and non-receptor tyrosine kinases in 187 high-risk B-progenitor ALL cases from the Children's Oncology Group P9906 cohort (25) identified 20 cases with somatic mutations in *JAK1*, *JAK2* and *JAK3* (53). The mutations were most commonly at or near R683 in the pseudokinase domain of JAK2, but were also found in the kinase domain of JAK2 and the pseudokinase domain of JAK1. Strikingly, the V617F mutation commonly observed in the myeloproliferative disorders (54-57) has not been identified in B-progenitor ALL, although the homolog of JAK2 V617F, JAK1 V658F, has been identified (58). The presence of JAK mutations was associated with *IKZF1* mutations, a BCR-ABL1-like gene expression profile, and poor outcome. Notably, JAK2 mutations (again, most commonly at R683 in the pseudokinase domain) had also recently been reported in up to one-quarter of cases of B-progenitor ALL associated with Down syndrome (59-61); however, most cases in the P9906 high-risk ALL cohort with JAK mutations were cases not associated with Down syndrome. JAK1 pseudokinase mutations have also been described in T-lineage ALL, albeit more commonly in adults than in children (58, 62). Like the JAK2 V617F mutation, the JAK1 and JAK2 mutations observed in ALL are transforming *in vitro*, conferring cytokine-independent growth and constitutive Jak-Stat activation when introduced into Ba/F3 cells (a murine pro-B cell line) expressing the erythropoietin or thrombopoietin receptors (53, 60, 63).

The Janus kinases are key mediators of hematopoietic cytokine receptor signal transduction (64-66). The identification of distinct JAK mutations in myeloproliferative diseases and ALL suggested that different mutated JAK alleles


A
DS-22
DS-21
DS-20
DS-16
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DS-9
0.108 Mb
PAR1
3.10 Mb
NT NT NT NT NT NT
B
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Chr X/Y (Mb)
2
-2
DS-16 Agilent
Agilent probes
CRLF2
IL3RA
SLC25A6
SFRS17A
CSF2RA
P2RY8
ASMT
C
Sample
H47-15
DS-21
H47-2
H47-18
Hypo-4
Hypo-9
NTC
Down Y Y N N N N
P2RY8-CRLF2
1.34kb
Actin
228bp
D
P2RY8 exon 1
CRLF2 exon 1
180
190
200
GCTGCTTCTGCAGGCATGGGGCGGCTGGTTCTG
M G R L V L
E
P2RY8-CRLF2 positive
JAK2 R683G positive
CD19 APC
Mouse IgG2a PE
CRLF2 PE
F
WT
683G
933R
JAK2
P2RY8-CRLF2
IL7R
IL7
IL3
pSTAT5
STAT5
pJAK2
PCNA
G
Ba/F3 transformation assays
Cells (x10^6/ml)
P2RY8-CRLF2
JAK2 WT
P2RY8-CRLF2, JAK2 WT
R683G
P2RY8-CRLF2, R683G
P933R
P2RY8-CRLF2, P933R
Days
H
REH
B7 P2C Jak2 933R
B7 P2C Jak2 683G
B7 P2C
B7 Jak2 683G
B7 Jak2 933R
B7
Cells (x10^6/ml)
[Jak inhibitor I] μM

Figure 1.

may interact with different downstream signaling pathways and influence the disease lineage. Recent studies have shown that the presence of JAK mutations in ALL are associated with chromosomal alterations, resulting in overexpression of the cytokine receptor CRLF2 (cytokine receptor-like factor 2, or TSLPR, thymic stromal lymphopoietin receptor), highlighting a new pathway of perturbed lymphoid signaling in ALL.

SNP array profiling of the high-risk pediatric ALL cohort described above demonstrated that many of the JAK-mutated cases harbored focal DNA copy number alterations, most commonly interstitial deletions, involving a cluster of hematopoietic cytokine receptor genes including *IL3RA* (interleukin 3 receptor alpha) and *CSF2RA* (GM-CSF receptor) at the pseudoautosomal region 1 (PAR1) at Xp/Yp. These alterations were adjacent to the *CRLF2* locus at PAR1, and were associated with markedly elevated expression of CRLF2 (67). Notably, Russell, Harrison and colleagues had also identified dysregulated expression of CRLF2 arising from rearrangement of *CRLF2* into the immunoglobulin heavy chain locus (*IGH@-CRLF2*), or associated with the PAR1 deletion, in a subset of B-progenitor ALL (68). The deletion extended from intron 1 of *P2RY8* (encoding the purinergic receptor gene P2Y, G-protein coupled, 8) to immediately upstream of the first coding exon of *CRLF2* (58). The deletion breakpoints were tightly clustered and resulted in a novel fusion transcript, *P2RY8-CRLF2*, in which the first, non-coding exon of *P2RY8* is fused to the entire coding region of CRLF2. *P2RY8* is a member of a family of purinergic receptor genes that is expressed in hematopoietic cells, including leukemic blasts, and has previously been identified as a rare target of translocation to *SOX5* in lymphoma (69).

CRLF2 alterations in B-progenitor ALL have been subsequently confirmed and identified by multiple groups, including adult ALL (58, 67, 68, 70, 71). *CRLF2* is rearranged in five to seven percent of B-progenitor childhood ALL cases, most commonly by *IGH@-CRLF2* rearrangement or the PAR1 deletion resulting in expression of *P2RY8-CRLF2*. Both alterations result in increased cell surface expression of CRLF2 by leukemic cells, and flow cytometric analysis of CRLF2 expression may be used to detect CRLF2-rearranged cases. Less commonly, *CRLF2* is rearranged to other, as yet unknown partner genes or harbors presumed activating mutations, most commonly F232C (70, 72). A striking observation is that *CRLF2* alteration, most commonly the PAR1 deletion, is present in over 50% of ALL associated with Down syndrome (DS-ALL) (58, 71), in which other chromosomal rearrangements characteristic of childhood ALL are uncommon (73). The basis for this increased frequency in DS-ALL is currently unknown.

In both DS- and non-DS-ALL, *CRLF2* rearrangement is significantly associated with the presence of activating Janus kinase mutations (58, 68, 70, 71). Over half of *CRLF2*-rearranged cases harbor activating *JAK1* or *JAK2* mutations, and conversely, nearly all JAK-mutated cases have *CRLF2* rearrangements, suggesting that these lesions together contribute to leukemogenesis. Importantly, in non-DS-ALL, CRLF2 alteration and JAK mutations are associated with the presence of *IKZF1* alterations, and several studies have observed strong associations between *CRLF2*/*JAK* alterations and very poor outcome (67, 74), suggesting that JAK inhibition may be a useful therapeutic approach in these high-risk cases that at present frequently fail maximal therapy. Importantly, however, the association between *CRLF2* alterations and poor outcome has not been observed in all cohorts (58), and the association with inferior outcome may be in part attributable to cohorts enriched for high-risk ALL cases, or cases of Hispanic/Latino ethnicity, which is associated with *CRLF2* rearrangement (67).

CRLF2 forms a heterodimeric receptor with interleukin-7 receptor alpha (IL7RA) for the cytokine TSLP (thymic stromal lymphopoietin) (75-77). TSLP/CRLF2 signaling has a role in dendritic cell development (78), T cell responses (79, 80), allergic inflammation (81-83), and promotes the proliferation of normal and leukemic B cells (84-88), but at present the requirement for CRLF2 signaling in normal B lymphoid ontogeny is unclear, and it may be dispensable (85, 89). The downstream mediators of TSLP/CRLF2 signaling are poorly defined and may differ between human and mouse, and activation of Jak-Stat signaling has been described for the human but not CRLF2 (89).

These differences may, in part, be due to limited homology of both the receptor and ligand across species.

Although the role of *CRLF2* in lymphopoiesis is incompletely understood, existing data suggests that aberrant CRLF2/JAK signaling contributes to leukemogenesis. Expression of either CRLF2 or mutant JAK alleles alone in Ba/F3 cells, a murine IL-3-dependent pro-B cell line widely used to examine the transforming effects of kinase mutations, usually does not result in transformation (58). A notable exception is JAK1 V658F, the homolog of JAK2 V617F, which transforms this cell line irrespective of cytokine receptor coexpression (58). Prior to the identification of *CRLF2* alterations in ALL, JAK mutations in ALL were shown (like the JAK2 V617F mutation observed in MPD) to transform Ba/F3 cells expressing the erythropoietin receptor (Ba/F3-EpoR cells) to cytokine-independent growth and result in constitutive Jak-Stat activation (53, 60, 63), suggesting that interaction of Jak mutants with a cytokine receptor scaffold is required for transformation. Subsequent studies have shown that coexpression of JAK mutations and CRLF2 in Ba/F3 cells is transforming, and that this transformation is inhibited by either pharmacologic JAK inhibition or short hairpin RNA-mediated knockdown of CRLF2 expression (58, 70, 71). Similarly, studies using primary murine hematopoietic progenitors have shown that enforced expression of CRLF2 alone promotes lymphoid expansion, but this is insufficient to result in the development of leukemia (ref (68) and unpublished data). Ongoing studies modeling CRLF2 dysregulation and JAK mutations will be of interest not only to determine the role of these alterations in leukemogenesis, but also to provide preclinical models of ALL that faithfully recapitulate human leukemia in which to test the efficacy of pharmacologic JAK inhibitors. This is particularly important as therapeutic JAK inhibition is now being pursued in other JAK-mutated disease, such as the myeloproliferative diseases (90, 91). Importantly, these studies must also model the effects of additional genetic lesions commonly observed in *CRLF2*/JAK-mutated ALL, including deletion or mutation of B-lymphoid transcriptional regulators such as *IKZF1* and *PAX5* and deletion of *CDKN2A*/*CDKN2B* (INK4/ARF). It will also be important to determine the potential utility of JAK inhibitors in BCR-ABL1-like and/or CRLF2-rearranged cases that lack JAK mutations but exhibit evidence of JAK-STAT pathway activation by gene expression profiling or flow cytometric analysis.

Future directions for genomic profiling in high-risk ALL

Integrated analysis of genomic data has been exceptionally informative in identifying novel genetic alterations in ALL; however, our understanding of the genetic basis of high-risk disease remains incomplete. For example, almost one-half of *CRLF2*-rearranged cases lack an activating JAK mutation, yet may have a BCR-ABL1-like gene expression profile, suggesting that additional cooperating or kinase-activating lesions remain to be identified. Moreover, many "BCR-ABL-like" cases lack *CRLF2* alterations, and the genetic alterations driving these leukemias remain unknown. Similarly, there remains a substantial proportion of ALL cases that lack known cytogenetic alterations and fail therapy, and the frequency of these cases rises with increasing age. Compared to childhood leukemia, there is a lack of detailed, high resolution genomic profiling data from adolescent and adult ALL (92-94), which has a markedly inferior outcome to that of childhood ALL. The frequency of Ph+ ALL rises progressively with increasing age, but this alone does not account for the poor outcome of ALL with increasing age, and at present it is unclear if the frequency of poor risk mutations and expression profiles observed in pediatric ALL will be recapitulated in the adult setting. This is a critical issue and a area of active enquiry. Furthermore, several high-risk subtypes of leukemia have either not been studied in detail (e.g. ALL with low hypodiploidy) (95-98) or have few structural genetic alterations on microarray analysis (e.g. *MLL*-rearranged leukemia) (13, 26). Also, while microarray platforms have provide important insights into DNA copy number alterations in ALL, they do not directly detect structural rearrangements or DNA sequence alterations.

Thus, future genomic profiling studies of ALL require detailed analysis of less well-studied

cohorts and the application of novel genomic profiling technologies that interrogate both genetic and epigenetic changes. Detailed candidate gene sequencing studies in ALL have identified new mutations in B-progenitor ALL (99), suggesting that genome-wide sequencing is required to identify the full complement of genetic alterations in this disease. This is now feasible with next-generation, massively parallel sequencing of tumor nucleic acids (100). Next-generation sequencing of either tumor DNA or RNA has identified new targets of mutation in AML (101, 102), T-lineage ALL (103), and lymphoma (104), and has identified new targets of rearrangement in cancer (105, 106), including B-lineage ALL (107). It is likely that as the time and cost requirements of these methods decline, sequencing-based approaches will assume greater importance in interrogating cancer genomes and may supplant array-based methodologies.

Acknowledgements

The authors thank colleagues at St Jude Children's Research Hospital and the Children's Oncology Group that have contributed to this work. Studies described were supported by ALSAC/St Jude and the National Institutes of Health. C.G.M. is supported by the American Society of Hematology, the American Association of Cancer Research, and is a Pew Scholar in the Biomedical Sciences.

References

1. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. *N Engl J Med* 2004;**350:**1535-1548.
2. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. *Lancet* 2008;**371:**1030-1043.
3. Pui CH, Pei D, Sandlund JT, Ribeiro RC, Rubnitz JE, Raimondi SC*, et al.* Long-term results of St Jude Total Therapy Studies 11, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. *Leukemia* 2009;**Epub ahead of print:**doi:10.1038/leu.2009.1252.
4. Pui CH, Campana D, Pei D, Bowman WP, Sandlund JT, Kaste SC*, et al.* Treating childhood acute lymphoblastic leukemia without cranial irradiation. *N Engl J Med* 2009;**360:**2730-2741.
5. Rowe JM, Buck G, Burnett AK, Chopra R, Wiernik PH, Richards SM*, et al.* Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. *Blood* 2005;**106:**3760-3767.
6. Fielding AK, Richards SM, Chopra R, Lazarus HM, Litzow MR, Buck G*, et al.* Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. *Blood* 2007;**109:**944-950.
7. Nguyen K, Devidas M, Cheng SC, La M, Raetz EA, Carroll WL*, et al.* Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study. *Leukemia* 2008;**22:**2142-2150.
8. Nachman J. Clinical characteristics, biologic features and outcome for young adult patients with acute lymphoblastic leukaemia. *Br J Haematol* 2005;**130:**166-173.
9. Stock W, La M, Sanford B, Bloomfield CD, Vardiman JW, Gaynon P*, et al.* What determines the outcomes for adolescents and young adults with acute lymphoblastic leukemia treated on cooperative group protocols? A comparison of Children's Cancer Group and Cancer and Leukemia Group B studies. *Blood* 2008;**112:**1646-1654.
10. Harrison CJ, Foroni L. Cytogenetics and molecular genetics of acute lymphoblastic leukemia. *Rev Clin Exp Hematol* 2002;**6:**91-113.
11. Harrison CJ. Cytogenetics of paediatric and adolescent acute lymphoblastic leukaemia. *Br J Haematol* 2009;**144:**147-156.
12. Gleissner B, Gokbuget N, Bartram CR, Janssen B, Rieder H, Janssen JW*, et al.* Leading prognostic relevance of the BCR-ABL translocation in adult acute B-lineage lymphoblastic leukemia: a prospective study of the German Multicenter Trial Group and confirmed polymerase chain reaction analysis. *Blood* 2002;**99:**1536-1543.
13. Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD*, et al.* Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* 2007;**446:**758-764.
14. Van Vlierberghe P, van Grotel M, Beverloo HB, Lee C, Helgason T, Buijs-Gladdines J*, et al.* The cryptic chromosomal deletion del(11)(p12p13) as a new activation mechanism of LMO2 in pediatric T-cell acute lymphoblastic leukemia. *Blood* 2006;**108:**3520-3529.
15. Balgobind BV, Van Vlierberghe P, van den Ouweland AM, Beverloo HB, Terlouw-Kromosoeto JN, van Wering ER*, et al.* Leukemia-associated NF1 inactivation in patients with pediatric T-ALL and AML lacking evidence for neurofibromatosis. *Blood* 2008;**111:**4322-4328.
16. Gutierrez A, Sanda T, Grebliunaite R, Carracedo A, Salmena L, Ahn Y*, et al.* High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. *Blood* 2009;**114:**647-650.
17. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J*, et al.* The landscape of somatic copy-number alteration across human cancers. *Nature* 2010;**463:**899-905.
18. Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J*, et al.* BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature* 2008;**453:**110-114.

19. Kuiper RP, Schoenmakers EF, van Reijmersdal SV, Hehir-Kwa JY, van Kessel AG, van Leeuwen FN*, et al.* High-resolution genomic profiling of childhood ALL reveals novel recurrent genetic lesions affecting pathways involved in lymphocyte differentiation and cell cycle progression. *Leukemia* 2007;**21:**1258-1266.

20. Kawamata N, Ogawa S, Zimmermann M, Kato M, Sanada M, Hemminki K*, et al.* Molecular allelokaryotyping of pediatric acute lymphoblastic leukemias by high-resolution single nucleotide polymorphism oligonucleotide genomic microarray. *Blood* 2008;**111:**776-784.

21. Nebral K, Denk D, Attarbaschi A, Konig M, Mann G, Haas OA*, et al.* Incidence and diversity of PAX5 fusion genes in childhood acute lymphoblastic leukemia. *Leukemia* 2008; doi:10.1038/leu.2008.1306.

22. Miller CB, Mullighan CG, Su X, Ma J, Wang M, Zhang J*, et al.* Pax5 Haploinsufficiency Cooperates with BCR-ABL1 to Induce Acute Lymphoblastic Leukemia. *Blood* 2008;**112:**abstract 293.

23. Dang J, Mullighan CG, Phillips LA, Mehta P, Downing JR. Retroviral and chemical mutagenesis identifies Pax5 as a tumor suppressor in B-progenitor acute lymphoblastic leukemia. *Blood* 2008;**112:**abstract 1798.

24. Collins-Underwood JR, Miller CB, Downing JR, Mullighan CG. Ikzf1 Haploinsufficiency Contributes to the Pathogenesis of BCR-ABL1 Positive Acute Lymphoblastic Leukemia. *Blood* 2009;**114:**abstract 678.

25. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB*, et al.* Deletion of IKZF1 and Prognosis in Acute Lymphoblastic Leukemia. *N Engl J Med* 2009;**360:**470-480.

26. Bardini M, Spinelli R, Bungaro S, Mangano E, Corral L, Cifola I*, et al.* DNA copy-number abnormalities do not occur in infant ALL with t(4;11)/MLL-AF4. *Leukemia* 2010;**24:**169-176.

27. Parker H, An Q, Barber K, Case M, Davies T, Konn Z*, et al.* The complex genomic profile of ETV6-RUNX1 positive acute lymphoblastic leukemia highlights a recurrent deletion of TBL1XR1. *Genes Chromosomes Cancer* 2008;**47:**1118-1125.

28. Iacobucci I, Storlazzi CT, Cilloni D, Lonetti A, Ottaviani E, Soverini S*, et al.* Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients: on behalf of Gruppo Italiano Malattie Ematologiche dell'Adulto Acute Leukemia Working Party (GIMEMA AL WP). *Blood* 2009;**114:**2159-2167.

29. Martinelli G, Iacobucci I, Storlazzi CT, Vignetti M, Paoloni F, Cilloni D*, et al.* IKZF1 (Ikaros) deletions in BCR-ABL1-positive acute lymphoblastic leukemia are associated with short disease-free survival and high rate of cumulative incidence of relapse: a GIMEMA AL WP report. *J Clin Oncol* 2009;**27:**5202-5207.

30. Georgopoulos K, Moore DD, Derfler B. Ikaros, an early lymphoid-specific transcription factor and a putative mediator for T cell commitment. *Science* 1992; **258:**808-812.

31. Georgopoulos K, Bigby M, Wang JH, Molnar A, Wu P, Winandy S*, et al.* The Ikaros gene is required for the development of all lymphoid lineages. *Cell* 1994; **79:**143-156.

32. Molnar A, Georgopoulos K. The Ikaros gene encodes a family of functionally diverse zinc finger DNA-binding proteins. *Mol Cell Biol* 1994;**14:**8292-8303.

33. Molnar A, Wu P, Largespada DA, Vortkamp A, Scherer S, Copeland NG*, et al.* The Ikaros gene encodes a family of lymphocyte-restricted zinc finger DNA binding proteins, highly conserved in human and mouse. *J Immunol* 1996;**156:**585-592.

34. Klug CA, Morrison SJ, Masek M, Hahm K, Smale ST, Weissman IL. Hematopoietic stem cells and lymphoid progenitors express different Ikaros isoforms, and Ikaros is localized to heterochromatin in immature lymphocytes. *Proc Natl Acad Sci U S A* 1998;**95:**657-662.

35. Georgopoulos K. Haematopoietic cell-fate decisions, chromatin regulation and ikaros. *Nat Rev Immunol* 2002;**2:**162-174.

36. Sun L, Goodman PA, Wood CM, Crotty ML, Sensel M, Sather H*, et al.* Expression of aberrantly spliced oncogenic ikaros isoforms in childhood acute lymphoblastic leukemia. *J Clin Oncol* 1999;**17:**3753-3766.

37. Sun L, Heerema N, Crotty L, Wu X, Navara C, Vassilev A*, et al.* Expression of dominant-negative and mutant isoforms of the antileukemic transcription factor Ikaros in infant acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A* 1999;**96:**680-685.

38. Iacobucci I, Lonetti A, Messa F, Cilloni D, Arruga F, Ottaviani E*, et al.* Expression of spliced oncogenic Ikaros isoforms in Philadelphia-positive acute lymphoblastic leukemia patients treated with tyrosine kinase inhibitors: implications for a new mechanism of resistance. *Blood* 2008;**112:**3847-3855.

39. Winandy S, Wu P, Georgopoulos K. A dominant mutation in the Ikaros gene leads to rapid development of leukemia and lymphoma. *Cell* 1995;**83:**289-299.

40. Tonnelle C, Bardin F, Maroc C, Imbert AM, Campa F, Dalloul A*, et al.* Forced expression of the Ikaros 6 isoform in human placental blood CD34(+) cells impairs their ability to differentiate toward the B-lymphoid lineage. *Blood* 2001;**98:**2673-2680.

41. Tonnelle C, Dijon M, Moreau T, Garulli C, Bardin F, Chabannon C. Stage specific over-expression of the dominant negative Ikaros 6 reveals distinct role of Ikaros throughout human B-cell differentiation. *Mol Immunol* 2009;**46:**1736-1743.

42. Trageser D, Iacobucci I, Nahar R, Duy C, von Levetzow G, Klemm L*, et al.* Pre-B cell receptor-mediated cell cycle arrest in Philadelphia chromosome-positive acute lymphoblastic leukemia requires IKAROS function. *J Exp Med* 2009;**206:**1739-1753.

43. Papaemmanuil E, Hosking FJ, Vijayakrishnan J, Price A, Olver B, Sheridan E*, et al.* Loci on 7p12.2, 10q21.2 and 14q11.2 are associated with risk of childhood acute lymphoblastic leukemia. *Nat Genet* 2009;**41:**1006-1010.

44. Trevino LR, Yang W, French D, Hunger SP, Carroll WL, Devidas M*, et al.* Germline genomic variants associated with childhood acute lymphoblastic leukemia. *Nat Genet* 2009;**41:**1001-1005.

45. Prasad RB, Hosking FJ, Vijayakrishnan J, Papaemmanuil E, Koehler R, Greaves M*, et al.* Verification of the susceptibility loci on 7p12.2, 10q21.2, and 14q11.2 in precursor B-cell acute lymphoblastic leukemia of childhood. *Blood* 2010;**115:**1765-1767.

46. Akasaka T, Balasas T, Russell LJ, Sugimoto KJ, Majid A, Walewska R*, et al.* Five members of the CEBP transcription factor family are targeted by recurrent IGH translocations in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). *Blood* 2007; **109:**3451-3461.

47. Chang LW, Payton JE, Yuan W, Ley TJ, Nagarajan R, Stormo GD. Computational identification of the normal and perturbed genetic networks involved in myeloid differentiation and acute promyelocytic leukemia. *Genome Biol* 2008;**9:**R38.

48. Kuiper RP, Waanders E, van der Velden VH, van Reijmersdal SV, Venkatachalam R, Scheijen B*, et al.* IKZF1 deletions predict relapse in uniformly treated pediatric precursor B-ALL. *Leukemia* 2010;**doi:10.1038/leu.2010.87**.

49. Mullighan CG, Phillips LA, Su X, Ma J, Miller CB, Shurtleff SA*, et al.* Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. *Science* 2008;**322:**1377-1380.

50. Yang JJ, Bhojwani D, Yang W, Cai X, Stocco G, Crews K*, et al.* Genome-wide copy number profiling reveals molecular evolution from diagnosis to relapse in childhood acute lymphoblastic leukemia. *Blood* 2008;**112:**4178-4183.

51. Kuiper RP, van Leeuwen FN, van der Velden V, van Reijmersdal SV, de Vries J, Keijzers-Vloet STM*, et al.* Ikaros Is a Frequently Affected Hematopoietic Differentiation Factor in Pediatric Relapse-Prone Precursor B-Cell Acute Lymphoblastic Leukemia. *Blood* 2008;**112:**abstract 4144.

52. Den Boer ML, van Slegtenhorst M, De Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST*, et al.* A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. *Lancet Oncol* 2009;**10:**125-134.

53. Mullighan CG, Zhang J, Harvey RC, Collins-Underwood JR, Schulman BA, Phillips LA*, et al.* JAK mutations in high-risk childhood acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A* 2009;**106:**9414-9418.

54. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C*, et al.* A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 2005;**434:**1144-1148.

55. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR*, et al.* A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005;**352:**1779-1790.

56. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ*, et al.* Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* 2005;**7:**387-397.

57. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S*, et al.* Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet* 2005;**365:**1054-1061.

58. Mullighan CG, Collins-Underwood JR, Phillips LA, Loudin MG, Liu W, Zhang J*, et al.* Rearrangement of CRLF2 in B-progenitor- and Down syndrome-associated acute lymphoblastic leukemia. *Nat Genet* 2009; **41:**1243-1246.

59. Malinge S, Ben-Abdelali R, Settegrana C, Radford-Weiss I, Debre M, Beldjord K*, et al.* Novel activating JAK2 mutation in a patient with Down syndrome and B-cell precursor acute lymphoblastic leukemia. *Blood* 2007;**109:**2202-2204.

60. Bercovich D, Ganmore I, Scott LM, Wainreb G, Birger Y, Elimelech A*, et al*. Mutations of *JAK2* in acute lymphoblastic leukaemias associated with Down's syndrome. *Lancet* 2008;**372:**1484-1492.

61. Kearney L, Gonzalez De Castro D, Yeung J, Procter J, Horsley SW, Eguchi-Ishimae M*, et al.* A specific JAK2 mutation (JAK2R683) and multiple gene deletions in Down syndrome acute lymphoblastic leukaemia. *Blood* 2008;**113:**646-648.

62. Flex E, Petrangeli V, Stella L, Chiaretti S, Hornakova T, Knoops L*, et al.* Somatically acquired JAK1 mutations in adult acute lymphoblastic leukemia. *J Exp Med* 2008;**205:**751-758.

63. Gaikwad A, Rye CL, Devidas M, Heerema NA, Carroll AJ, Izraeli S*, et al.* Prevalence and clinical correlates of JAK2 mutations in Down syndrome acute lymphoblastic leukaemia. *Br J Haematol* 2009;**144:**930-932.

64. Baker SJ, Rane SG, Reddy EP. Hematopoietic cytokine receptor signaling. *Oncogene* 2007;**26:**6724-6737.

65. Ihle JN, Gilliland DG. Jak2: normal function and role in hematopoietic disorders. *Curr Opin Genet Dev* 2007;**17:**8-14.

66. Vainchenker W, Dusa A, Constantinescu SN. JAKs in pathology: Role of Janus kinases in hematopoietic malignancies and immunodeficiencies. *Semin Cell Dev Biol* 2008;**19:**385-393.

67. Harvey RC, Mullighan CG, Chen IM, Wharton W, Mikhail FM, Carroll AJ*, et al*. Rearrangement of *CRLF2* is associated with mutation of *JAK* kinases, alteration of *IKZF1*, Hispanic/Latino ethnicity and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. *Blood* 2010;**in press**.

68. Russell LJ, Capasso M, Vater I, Akasaka T, Bernard OA, Calasanz MJ, *et al.* Deregulated expression of cytokine receptor gene, CRLF2, is involved in lymphoid transformation in B-cell precursor acute lymphoblastic leukemia. *Blood* 2009;**114:**2688-2698.

69. Storlazzi CT, Albano F, Lo Cunsolo C, Doglioni C, Guastadisegni MC, Impera L, *et al.* Upregulation of the SOX5 by promoter swapping with the P2RY8 gene in primary splenic follicular lymphoma. *Leukemia* 2007;**21:**2221-2225.

70. Yoda A, Yoda Y, Chiaretti S, Bar-Natan M, Mani K, Rodig SJ, *et al.* Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. *Proc Natl Acad Sci U S A* 2010;**107:**252-257.

71. Hertzberg L, Vendramini E, Ganmore I, Cazzaniga G, Schmitz M, Chalker J, *et al.* Down syndrome acute lymphoblastic leukemia: a highly heterogeneous disease in which aberrant expression of CRLF2 is associated with mutated JAK2: a report from the iBFM Study Group. *Blood* 2010;**115:**1006-1017.

72. Chapiro E, Russell L, Lainey E, Kaltenbach S, Ragu C, Della-Valle V, *et al.* Activating mutation in the TSLPR gene in B-cell precursor lymphoblastic leukemia. *Leukemia* 2010;**24:**642-645.

73. Forestier E, Izraeli S, Beverloo B, Haas O, Pession A, Michalova K, *et al.* Cytogenetic features of acute lymphoblastic and myeloid leukemias in pediatric patients with Down syndrome: an iBFM-SG study. *Blood* 2008;**111:**1575-1583.

74. Cario G, Zimmermann M, Romey R, Gesk S, Vater I, Harbott J, *et al.* Presence of the P2RY8-CRLF2 rearrangement is associated with a poor prognosis in non-high-risk precursor B-cell acute lymphoblastic leukemia in children treated according to the ALL-BFM 2000 protocol. *Blood* 2010;**Epub doi:10.1182/blood-2009-11-256131**.

75. Pandey A, Ozaki K, Baumann H, Levin SD, Puel A, Farr AG, *et al.* Cloning of a receptor subunit required for signaling by thymic stromal lymphopoietin. *Nat Immunol* 2000;**1:**59-64.

76. Hiroyama T, Iwama A, Morita Y, Nakamura Y, Shibuya A, Nakauchi H. Molecular cloning and characterization of CRLM-2, a novel type I cytokine receptor preferentially expressed in hematopoietic cells. *Biochem Biophys Res Commun* 2000;**272:**224-229.

77. Park LS, Martin U, Garka K, Gliniak B, Di Santo JP, Muller W, *et al.* Cloning of the murine thymic stromal lymphopoietin (TSLP) receptor: Formation of a functional heteromeric complex requires interleukin 7 receptor. *J Exp Med* 2000;**192:**659-670.

78. Zhang W, Wang J, Wang Q, Chen G, Zhang J, Chen T, *et al.* Identification of a novel type I cytokine receptor CRL2 preferentially expressed by human dendritic cells and activated monocytes. *Biochem Biophys Res Commun* 2001;**281:**878-883.

79. Mazzucchelli R, Hixon JA, Spolski R, Chen X, Li WQ, Hall VL, *et al.* Development of regulatory T cells requires IL-7Ralpha stimulation by IL-7 or TSLP. *Blood* 2008;**112:**3283-3292.

80. Ziegler SF, Liu YJ. Thymic stromal lymphopoietin in normal and pathogenic T cell development and function. *Nat Immunol* 2006;**7:**709-714.

81. Al-Shami A, Spolski R, Kelly J, Keane-Myers A, Leonard WJ. A role for TSLP in the development of inflammation in an asthma model. *J Exp Med* 2005;**202:**829-839.

82. Rochman Y, Leonard WJ. Thymic stromal lymphopoietin: a new cytokine in asthma. *Curr Opin Pharmacol* 2008;**8:**249-254.

83. Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, Lewis DB, *et al.* Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005;**6:**1047-1053.

84. Ray RJ, Furlonger C, Williams DE, Paige CJ. Characterization of thymic stromal-derived lymphopoietin (TSLP) in murine B cell development in vitro. *Eur J Immunol* 1996;**26:**10-16.

85. Vosshenrich CA, Cumano A, Muller W, Di Santo JP, Vieira P. Pre-B cell receptor expression is necessary for thymic stromal lymphopoietin responsiveness in the bone marrow but not in the liver environment. *Proc Natl Acad Sci U S A* 2004;**101:**11070-11075.

86. Astrakhan A, Omori M, Nguyen T, Becker-Herman S, Iseki M, Aye T, *et al.* Local increase in thymic stromal lymphopoietin induces systemic alterations in B cell development. *Nat Immunol* 2007;**8:**522-531.

87. Brown VI, Hulitt J, Fish J, Sheen C, Bruno M, Xu Q, *et al.* Thymic stromal-derived lymphopoietin induces proliferation of pre-B leukemia and antagonizes mTOR inhibitors, suggesting a role for interleukin-7Ralpha signaling. *Cancer Res* 2007;**67:**9963-9970.

88. Scheeren FA, van Lent AU, Nagasawa M, Weijer K, Spits H, Legrand N, *et al.* Thymic stromal lymphopoietin induces early human B-cell proliferation and differentiation. *Eur J Immunol* 2010;**40:**955-965.

89. Carpino N, Thierfelder WE, Chang MS, Saris C, Turner SJ, Ziegler SF, *et al.* Absence of an essential role for thymic stromal lymphopoietin receptor in murine B-cell development. *Mol Cell Biol* 2004;**24:**2584-2592.

90. Pardanani A. JAK2 inhibitor therapy in myeloproliferative disorders: rationale, preclinical studies and ongoing clinical trials. *Leukemia* 2008;**22:**23-30.

91. Verstovsek S. Therapeutic potential of JAK2 inhibitors. *Hematology Am Soc Hematol Educ Program* 2009;636-642.

92. Paulsson K, Cazier JB, Macdougall F, Stevens J, Stasevich I, Vrcelj N, *et al.* Microdeletions are a general feature of adult and adolescent acute lymphoblastic leukemia: Unexpected similarities with pediatric disease. *Proc Natl Acad Sci U S A* 2008;**105:**6708-6713.

93. Usvasalo A, Elonen E, Saarinen-Pihkala UM, Raty R, Harila-Saari A, Koistinen P*, et al.* Prognostic classification of patients with acute lymphoblastic leukemia by using gene copy number profiles identified from array-based comparative genomic hybridization data. *Leukemia Res* 2010;**in press**.

94. Okamoto R, Ogawa S, Nowak D, Kawamata N, Akagi T, Kato M*, et al.* Genomic profiling of adult acute lymphoblastic leukemia (ALL) by single nucleotide polymorphism oligonucleotide microarray and comparison to pediatric ALL. *Haematologica* 2010;**in press. doi:10.3324/haematol.2009.011114**.

95. Harrison CJ, Moorman AV, Broadfield ZJ, Cheung KL, Harris RL, Reza Jalali G*, et al.* Three distinct subgroups of hypodiploidy in acute lymphoblastic leukaemia. *Br J Haematol* 2004;**125:**552-559.

96. Heerema NA, Nachman JB, Sather HN, Sensel MG, Lee MK, Hutchinson R*, et al.* Hypodiploidy with less than 45 chromosomes confers adverse risk in childhood acute lymphoblastic leukemia: a report from the children's cancer group. *Blood* 1999;**94:**4036-4045.

97. Pui CH, Williams DL, Raimondi SC, Rivera GK, Look AT, Dodge RK*, et al.* Hypodiploidy is associated with a poor prognosis in childhood acute lymphoblastic leukemia. *Blood* 1987;**70:**247-253.

98. Raimondi SC, Zhou Y, Mathew S, Shurtleff SA, Sandlund JT, Rivera GK*, et al.* Reassessment of the prognostic significance of hypodiploidy in pediatric patients with acute lymphoblastic leukemia. *Cancer* 2003; **98:**2715-2722.

99. Zhang J, Mullighan CG, Harvey RC, Buetow KE, Carroll WL, Chen I-M*, et al.* Mutations in the RAS Signaling, B-Cell Development, TP53/RB1, and JAK Signaling Pathways Are Common in High Risk B-Precursor Childhood Acute Lymphoblastic Leukemia (ALL): A Report From the Children's Oncology Group (COG) High-Risk (HR) ALL TARGET Project. *Blood* 2009;**114:**abstract 85.

100. Mardis ER, Wilson RK. Cancer genome sequencing: a review. *Hum Mol Genet* 2009;**18:**R163-168.

101. Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K*, et al.* DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature* 2008; **456:**66-72.

102. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K*, et al.* Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med* 2009;**361:**1058-1066.

103. Van Vlierberghe P, Palomero T, Khiabanian H, Van der Meulen J, Castillo M, Van Roy N*, et al.* PHF6 mutations in T-cell acute lymphoblastic leukemia. *Nat Genet* 2010;**42:**338-342.

104. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R*, et al.* Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet* 2010;**42:**181-185.

105. Maher CA, Kumar-Sinha C, Cao X, Kalyana-Sundaram S, Han B, Jing X*, et al.* Transcriptome sequencing to detect gene fusions in cancer. *Nature* 2009;**458:**97-101.

106. Maher CA, Palanisamy N, Brenner JC, Cao X, Kalyana-Sundaram S, Luo S*, et al.* Chimeric transcript discovery by paired-end transcriptome sequencing. *Proc Natl Acad Sci U S A* 2009;**106:**12353-12358.

107. Mullighan CG, Morin RD, Zhang J, Hirst M, Zhao Y, Yan C*, et al.* Next Generation Transcriptomic Resequencing Identifies Novel Genetic Alterations in High-Risk (HR) Childhood Acute Lymphoblastic Leukemia (ALL): A Report From the Children's Oncology Group (COG) HR ALL TARGET Project. *Blood* 2009;**114:**abstract 704.

INTERNATIONAL SOCIETY
OF PAEDIATRIC ONCOLOGY

T-ALL Molecular Pathogenesis: an Update

A. Thomas Look

Abstract

Subsets of childhood T-cell leukemias arise from oncogenes activated by antigen receptor gene translocations. Otherwise, little is known about the molecular pathogenesis of this thymic cancer. Here we show that three different T-cell oncogenes (LYL1, HOX11 and TAL1) are often expressed in the absence of chromosomal abnormalities, and that HOX11 activation is significantly associated with a favorable prognosis. Using oligonucleotide microarrays, we identified three distinct gene expression signatures that were indicative of leukemic arrest at specific stages of normal thymocyte development: LYL1+ (pre-T), HOX11+ (early cortical thymocyte) and TAL1+ (late cortical thymocyte). Hierarchical clustering analysis of the microarray findings allowed us to devise a prognostically relevant classification system that accommodated all T-cell cases in this series and integrated oncogene activation and specific chromosomal deletions into emerging multistep molecular pathways of thymocyte leukemogenesis. These results demonstrate a previously undetected molecular heterogeneity among childhood T-cell leukemias, and suggest the ability of gene expression profiling to stratify patients into clinically relevant subgroups.

Chromosomal translocations that produce a truncated and activated form of the *NOTCH1* receptor have been identified in rare cases of human T-cell acute lymphoblastic leukemia (T-ALL). To uncover more frequent types of *NOTCH1*-activating mutations, we tested several T-cell leukemia cell lines with a small molecule NOTCH pathway inhibitor and found evidence of cell-cycle arrest that could be rescued by introducing the intracellular NOTCH-1 domain. By resequencing portions of the *NOTCH1* genes in these cell lines, we were able to identify specific mutations affecting both the heterodimerization and PEST domains of NOTCH1, which caused increased NOTCH1 signaling. We then analyzed primary T-ALL samples and identified NOTCH1 mis-sense mutations within the heterodimerization domain (HD) in 27% and truncating mutations that deleted the PEST destruction box (DPEST) in 15% of childhood T-ALL blasts. Both of these regions were simultaneously mutated in the same NOTCH1 gene of 16% of cases, providing evidence for multi-hit mutagenesis affecting a single oncogene in primary T-ALL samples at diagnosis. Only 42% of cases had unmutated NOTCH1 genes. These mutations were shown to occur in each of the five multistep molecular pathways that can lead to the transformation of T-cell progenitors during development, suggesting that some form of NOTCH pathway disruption may be required as a first step in the induction of T-ALL regardless of the additional genes that ultimately become mutated. These findings greatly expand the role of activated NOTCH1 in the molecular pathogenesis of human T-ALL, and provide a strong rationale in for targeted therapies of this disease that interfere with NOTCH signaling, because mutationally activated forms of NOTCH1 are still dependent on enzymatic cleavage for activity.

Leukemias of T-cell precursors are identified and classified according to the expression of T-cell-associated surface antigens which are expressed during normal thymocyte differentiation[1]. In this tightly regulated process, the earliest T-cell precursors are characterized by the lack of expression of CD4 and CD8 surface markers. These double-negative thymocytes express CD7, TdT and cytoplasmic CD3, and proceed through four different stages of development (DN1 to DN4) defined by the expression of CD44 and CD25, after which the TCRb gene becomes rearranged, driving the production of intermediate single-positive cells

(ISPs) with a surface phenotype of $CD4^+$, $CD8^-$, $CD3^-$ that differentiate into early double-positive ($CD4^+$, $CD8^+$) cells. Subsequently, these DP progenitors acquire surface CD1 and differentiate into late cortical thymocytes showing a loss of CD1 and a gain of surface CD3 expression. This T-cell developmental process ends when mature $CD4^+$ or $CD8^+$ single-positive cells are produced[2].

The clinical features most closely associated with T-cell ALL are high blood leukocyte counts, a predominance in boys and men, central nervous system involvement, and radiographic evidence of a thymic mass in about one-half of cases at presentation. Historically, patients with T-cell ALL have had an adverse prognosis by comparison to patients with B-lineage ALL, but this gap has narrowed with wider use of intensive chemotherapy[3, 4]. Some authors contend that expression of specific antigens, including CD1 and/or coexpression of CD4 and CD8, CD2, CD5, CD10, or the coexpression of six or more T-cell markers may identify subgroups of T-cell ALL patients with better responses to therapy[3-7], but this argument remains controversial.

The human antigen-specific TCR molecule is a heterodimer composed of disulfide-linked a and b polypeptide subunits, each encoded by gene families containing variable, joining and constant sequence elements that rearrange at the DNA level to generate diversity, in a manner analogous to the *IG* genes. Hence, rearrangement of the *TCRa/b* genes can be used to establish clonality and lineage derivation within leukemias of T-cell progenitors.

Dysregulated expression of oncogenic transcription factors

BHLH, LIM and HOX Genes in T-ALL. In leukemias with a T-cell phenotype, chromosomal breakpoints consistently involve the *TCR* enhancer (7q34) or the *TCRa/d* enhancer (14q11), both of which are highly active in committed T-cell progenitors and can cause dysregulated expression of transcription factor genes located at the breakpoint on the reciprocal chromosome involved in these phenotype-specific rearrangements[8]. The affected transcription factors include: (i) genes encoding basic helix-loop-helix (bHLH) family members, such as *TAL1*[9, 10], *TAL2*[11], *LYL1*[12], *MYC*[13-15], and *BHLHB1*[16]; (ii) LIM-only domain (*LMO*) genes, such as *LMO1* and *LMO2*[17-19]; and (iii) the orphan homeobox genes *HOX11* and *HOX11L2*[20-26]. The observation that T-ALL oncogenes act as master transcriptional regulators during the embryonic development of specific organ systems suggests that their aberrant expression in T-cell precursors may contribute to the onset of leukemia by disrupting the mechanisms that control cell proliferation, differentiation and survival during the discrete steps of normal T-cell development.

The best characterized of these genes is *TAL1*, which is altered by the t(1;14) or by site-specific deletions in approximately one-fourth of childhood T-ALL cases[27-32]. *TAL1* is aberrantly expressed in the leukemic cells of 60% of children and 45% of adults with T-ALL (Fig. 2). TAL1 acts as a master regulatory protein during early hematopoietic development and is required for the generation of all blood cell lineages[33, 34], however, it does not seem to be required for the generation and function of hemopoietic stem cells during adult hematopoiesis[35]. This class II basic helix-loop-helix (bHLH) transcription factor binds to DNA by forming heterodimers with class I bHLH factors such as E2A and HEB[36]. The observation that loss of E2A function induces T-cell leukemias in mice[37, 34], and that the DNA-binding domain of TAL1 is dispensable for transformation[39] in transgenic mouse models, supports the notion that TAL1 mediated inhibition of E2A plays a critical role in the pathogenesis of T-ALL. A recent study demonstrating accelerated leukemogenesis in TAL1 transgenic mice on a E2A or HEB heterozygous background shows that inhibition of HEB as well as E2A contributes to transformation by TAL1[40].

The LIM-only domain genes, *LMO1/RBTN1/TTG1* and *LMO2/RBTN2/TTG2*[17-19], encode proteins that contain cysteine-rich LIM domains involved in protein-protein interactions. LMO2 interacts with TAL1 in erythroid cells as part of a pentameric complex that also includes E47, GATA1 and Ldb1[41-43]. Moreover, homozygous disruption of *LMO2* in mice causes the same phenotype as described above for *TAL1* knockout mice, indicating that the multiprotein complex involving LMO2 and TAL1 is required for normal hematopoietic development[44, 45]. In addition, overexpression of *LMO1* or *LMO2* in

thymocytes of transgenic mice leads to T-cell lymphomas[46-50], and accelerates the onset of leukemias in *TAL1* transgenic mice[51, 52]. Activation of LMO2 is also implicated in gene therapy induced T-ALL that occurred in two patients in a recent gene therapy clinical trial for X-linked severe combined immunodeficiency[53]. In both patients, the retroviral vector inserted near the *LMO2* gene resulting in overexpression of LMO2[54].

The homeodomain gene *HOX11* was originally isolated from the recurrent t(10;14)(q24;q11) in T-ALL[20, 22, 24&25] and is aberrantly expressed in 3% to 5% of pediatric and up to 30% of adult T-ALL cases (Fig. 1)[21, 55, 56]. Like other *HOX* genes, *HOX11* plays an important role in embryonic development, and functions as a master transcriptional regulator necessary for the genesis of the spleen[57, 58]. In the mouse embryo, *Hox11* expression can be detected in the branchial arches, restricted areas of the hindbrain and the splenic primordium[59, 60], where it is required for the survival of early splenic progenitors[58]. The proposed function of HOX11 as a transcriptional regulator, is supported by the presence of both a 61-amino acid, helix-turn-helix DNA-binding domain (or homeodomain) and by the localization of the HOX11 protein in the cell nucleus[26]. Recent data suggests that HOX11 may contribute to T cell transformation by blocking T cell differentiation and deregulating the cell cycle by blocking PP1/PP2A phosphatase activity[61, 62].

A second *HOX11* family member, *HOX11L2*, has been implicated in the pathogenesis of human T-ALL through characterization of the t(5;14)(q35;q32), a cryptic chromosomal rearrangement detectable only by fluorescence *in situ* hybridization and by chromosome painting techniques[23]. This translocation leads to the etopic expression of *HOX11L2*, possibly by bringing it under the influence of regulatory elements in the *CTIP2/BCL11B* gene, which is highly expressed during T-cell differentiation. In contrast to the predominance of *HOX11* expression in adult T-ALL cases, both the t(5;14) and expression of *HOX11L2* can be detected in 20% to 25% of children but in only 5% of adults with T-ALL (Figure 1)[21, 56, 63, 64]. The role of HOX11L2 as a master transcriptional regulator upstream of important pathways involved in cell fate determination is supported by its importance during embryonic development. In mice, *Hox11l2* expression is essential for normal development of the ventral medullary respiratory center. As a result *Hox11l2* deficient mice die soon after birth due to respiratory failure that resembles congenital central hypoventilation syndrome in humans[65].

HOX11 and HOX11L2 are closely related in structure, and have a high degree of homology at the amino acid level, especially in the homeobox domain, where their sequences differ by only three amino acids. The high level of structural homology in their DNA-binding domains supports the hypothesis that HOX11 and HOX11L2 may induce T-ALL through regulation of the same transcriptional targets, however, activation of *HOX11* and *HOX11L2* seem to be associated with clinically relevant differences that may result, at least in part, from differences in their mechanisms of action. The expression of *HOX11* is associated with a favorable prognosis both in children and in adults[2, 54, 101], while expression of *HOX11L2* has been associated with a high incidence of relapse in children with T-ALL[75, 63]. A new recurrent translocation has been recognized that targets and dysregualtes expression from the whole HOXA cluster. Gene expression analysis demonstrates that this subgroup shares aspects of the gene expression signature characteristic of *HOX11*- and *HOX11L2*-overexpressing T-ALLs[66].

Recently, the analysis of gene expression profiling using oligonucleotide microarrays has shown that the expression of different transcription factor oncogenes such as *TAL1, LYL1, HOX11* and *HOX11L2* is associated with distinct gene expression profiles. These unique signatures resemble those of thymocytes blocked at discrete stages of T-cell development (Fig. 2) and suggest that transcription factor oncogenes contribute to the pathogenesis of T-ALL by interfering with critical regulatory networks that control cell proliferation, survival and differentiation during T-cell development[75]. Although *TAL1*, *LMO2* and *HOX11* are all involved in translocations with the T cell receptor locus, all three of these genes have been shown to be overexpressed in cases in which no translocation in detected. A recent study has

shown that these genes can be biallelically activated suggesting that in some leukemias there is a mutation in a pathway that normally down-regulates these genes during T cell differentiation[67].

CALM-AF10 fusion gene in T-ALL. The t(10;11)(p13;q14) detected in approximately 3%-10% of T-ALL cases and in occasional AML cases results in the fusion of *CALM*, encoding a protein with high homology to the murine clathrin assembly protein ap3; with *AF10,* a gene identified in as an MLL partner in the MLL-AF10 fusion resulting from the t(10;11)(p13;q23)[68]. Although the mechanism of action of CALM-AF10 is still poorly understood, the expression of this fusion transcript has been associated with early arrest in T-cell development and to differentiation into the gamma-delta lineage in T-ALL[69]. Microarray expression analysis has revealed that CALM-AF10 cases overexpress *HOXA* genes and the oncogene *BMI1* that suppresses the p16 and p19 cell cycle inhibitors[70].

MLL-ENL fusion genes in T-ALL. Microarray gene expression analysis of *MLL*-rearranged B-lineage leukemias has shown that these tumors have a characteristic gene expression signature that includes the upregulation of several *HOX* genes and the expression of numerous myeloid markers[71, 72]. Both early B- and T-cell ALLs with *MLL* rearrangements showed a characteristic upregulation of specific *HOX* genes including *HOXA9, HOXA10, HOXC6* and the *HOX* gene regulator *MEIS1*[71-73]. These results, together with the demonstration that HOXA9 is required for the transformation of hemopoietic precursors by MLL fusion oncogenes in murine leukemia models[74], emphasize the central role of *HOX* gene dysregulation in the pathogenesis of *MLL*-rearranged leukemias.

NOTCH1 mutations

The *TAN1* gene, which shares homology with the *Drosophila notch* gene, is involved in the t(7;9) translocation leading to its relocation to the *TCRB* locus and dysregulated expression[76]. This translocation is very rare, occurring in less than 1% of T-ALLs. Bone marrow reconstitution experiments in the mouse have demonstrated that similar forms of activated NOTCH1 are potent inducers of T-ALL[77]. Although the mechanism through which NOTCH1 signaling promotes T-ALL is not known, NOTCH1 has been shown to play essential roles in normal T cell development, most notably at the level of T cell commitment[78]. NOTCH1 has also been shown to inhibit the transcription factor E47 that is essential for both B and T cell development[79]. Therefore, NOTCH1 may contribute to leukemogenesis by altering T development through inhibition of E47.

Recent studies have demonstrated a broader involvement of *NOTCH1* in T-ALL. Activating mutations in *NOTCH1* were detected in over 50% of T-ALL patient samples[80] (Fig. 3). The mutations were detected in all subtypes of T-ALL and were found in two regions of the NOTCH1 protein. Missense mutations in the heterodimerization domain activate NOTCH signaling by altering the interaction between the transmembrane subunit and the inhibitory extracellular subunit of NOTCH1. In addition, frameshift and point mutations that introduce premature stop codons are observed, which delete the C-terminal PEST destruction sequences and thereby increase ICN1 stability and signaling activity[81].

The *NOTCH* genes encode single pass transmembrane receptors that regulate apoptosis, proliferation and cell fate determination in multicellular organisms. Pro-NOTCH1 is cleaved to produce a NOTCH1 heterodimer that is presented on the cell surface. Binding of NOTCH ligands, such as Delta and Serrate, initiates a series of additional proteolytic cleavages in NOTCH1. The last of these cleavages, which is catalyzed by g-secretase, results in the release of the intracellular domain of NOTCH1 (ICN), permitting it to translocate to the nucleus and form part of a multiprotein complex that regulates gene transcription (Fig. 4). Treatment of T-ALL cell lines with mutations in *NOTCH1* with g-secretase inhibitors led to G_0/G_1 arrest demonstrating that the cells are dependent on NOTCH signaling for growth and suggesting that activation of NOTCH may contribute to the pathogenesis of T-ALL by promoting cell cycle progression[80]. The g-secretase enzyme also cleaves the amyloid precursor protein leading to the production of plaques in Alzheimer's patients. As a result, g-secretase inhibitors have already been developed for use as drugs. Clinical trials are

currently underway to determine if NOTCH pathway inhibitors will be efficacious in treating patients with T-ALL.

Other genes activated by translocation. Transcription factors are not the only genes activated by translocation to the sites of the *IG* or *TCR* genes. In cases of B-precursor ALL carrying the t(5;14), for example, the *IL-3* gene is activated by juxtaposition with the *IG* heavy-chain locus[82, 83]. In T-cell ALL, relocation to the *TCRB* locus activates expression of the *LCK* tyrosine kinase genes in cases with the t(1;7)[84-86].

Recently, a unique fusion gene resulting in ABL kinase activation has been identified in T-ALL. This fusion results from a small deletion that removes an approximately 500 kb segment of chromosome 9 with breakpoints within one of the introns of the *NUP214* gene and within the first intron of *ABL*. This deleted fragment is ligated as a circular episome that encodes a fusion gene between amino terminal sequences of NUP214 and the ABL kinase. It is maintained and amplified as an episomal structure lacking a centrosome, and is small enough that it does not appear as a double-minute chromatin body and can only be visualized cytogenetically by FISH analysis for the affected genes. The NUP214-ABL fusion occurs in the subset of cases with activated HOX11 or HOX11L2 homeobox transcription factors[87].

Tumor Suppressor Genes

Loss of function of a tumor suppressor protein, occurring through deletion or mutational inactivation of both chromosomal loci of the gene that encodes it, leads to malignant transformation. Knudson first proposed that inactivation of both alleles of a single locus is needed to initiate the development of retinoblastoma, basing his ideas on the observed frequencies of hereditary and sporadic forms of this disease[88]. Allelic loss of defined regions of many different chromosomes has been linked to specific types of human tumors. By analogy with the findings in retinoblastoma, a reasonable hypothesis is that each of these regions harbors a tumor suppressor gene whose product is uniquely involved in the inhibition of cell cycle progression and promotion of terminal differentiation of the normal cells that give rise to these different types of tumors. Tumor suppressor genes that play an important role in ALL include *p53*and the *p16* locus.

p53, located on chromosome 17, band p13, is mutated or lost through chromosomal deletion in a wide variety of human tumors[89], including colon cancer, lung cancer, breast cancer, and osteosarcoma. Families with Li-Fraumeni syndrome, which predisposes to a variety of cancers including sarcomas, brain tumors, leukemias, adrenocortical carcinomas and premenopausal breast cancers, have germline mutations in the *p53* gene[90-93]. *p53* encodes a 53-kDa transcription factor that functions as a cell cycle and apoptosis checkpoint regulator[89, 94-98]. The p53 protein is increased by DNA damage, blocks cell division at G_1 to allow DNA repair, and activates apoptosis in cells that have sustained DNA damage[99-105]. The mechanism of p53 activation is triggered by the loss of activity of MDM2 after DNA damage (via ATM) or oncogenic stress (via p14/ARF). As a negative regulator of p53, MDM2 induces the ubiqitination of p53 and its degradation by the proteasome. Hence, when MDM2 activity is abolished, p53 accumulates and certain cell cycle regulatory genes such as *p21(WAF1/CIP1/SDI1/CAP20)* and proapoptotic factor genes such as *BAX, PUMA* and *NOXA* are transcriptionally activated.

p53 is also inactivated in a variety of hematopoietic malignancies, including B-cell ALL and Burkitt's lymphoma, but is mutated or deleted in less than 3% of pediatric B-precursor or T-cell ALL cases at diagnosis[105-107]. It thus appears to play a limited role in the etiology of pediatric leukemia. However, *p53* mutations are seen in approximately 25% of relapsed T-cell ALL cases, suggesting a role for *p53* inactivation in the development of resistant disease[105, 106]. In addition, *p53* mutations were detected in 3 of 10 ALL patients who failed on induction therapy or suffered early relapse, further supporting a role for *p53* inactivation in disease progression[108, 109].

The cyclin-dependent kinase inhibitors (CDKIs), which include p15 (INK4B/MTS2), p16 (INK4A/MTS1/CDKN2), p18 (INK4C), p19 (INK4D), p21 (WAF1/CIP1/SDI1/CAP20), p27 (KIP1), and p57 (KIP2) constitute a family of tumor suppressors that negatively regulate the cell cycle by inhibiting cyclin-dependent kinase (CDK) phosphorylation of pRB[110]. The *INK4A* locus,

located on the short arm of chromosome band 9q21, contains two different tumor suppressor genes, *p16INK4A* and *p14ARF* (*p19ARF* in mice)[111, 112], each with a distinct promoter and first exon but common second and third exons. Despite this close relationship at the genomic level, p16 and p14 have totally unrelated amino acid sequences as they use different reading frames in their common second and third exons[113]. A third tumor suppressor gene, the cyclin-dependent kinase inhibitor *p15INK4B*, also resides in this region[114, 115]. p16INK4A and p15INK4B directly inhibit cyclin D-CDK4/6 complexes and interfere with cell cycle progression Cyclin D-CDK4/6 complexes promote entry into S phase through phosphorylation of the retinoblastoma protein, $_{P}$RB, leading to the release of transcription factors, such as E2F, that promote entry into S phase. By contrast, p14ARF, lacks a direct effect on the cell cycle machinery acting instead to stabilize and upregulate p53 through the inhibition of MDM2[115-118]. The role of the *INK4a* locus in tumorigenesis was confirmed by selective targeted deletion of *p16* and *p19* in mice. *p16* deficient mice (with intact *p19*) as well as *p19* deficient mice (with intact *p16*) develop tumors (primarily lymphomas and fibrosarcomas)[111, 119, 120].

The short arm of chromosome 9 is the most frequent target of chromosomal alterations in human cancer. In particular, human leukemias and lymphomas show a high frequency of 9p21 deletions involving both the *p16INK4A*/*p14ARF*and the *p15INK4B* loci. Epigenetic silencing of these tumor suppressor genes through hypermethylation of their promoter sequences represents an alternative mechanism of gene inactivation. While *p16INK4A*/*p14ARF* and *p15INK4B* are homozygously deleted in 20% to 30% of B-precursor ALL cases and in 70% to 80% of T-cell ALL cases, epigenetic silencing of the *p15INK4B* promoter has been observed in 44% of primary B-lineage ALLs[121-133].

The clinical impact of *p15INK4B* and *p16INK4A*/*p14ARF* deletions in ALL remains controversial. On the one hand, homozygous *p16* deletion is related to high-risk features at diagnosis and to an increased risk of relapse and death in childhood ALL[132, 134, 135], while on the other, *p15INK4B* and *p16INK4A*/*p14ARF* deletions were not associated with clinical outcome in a study of adult ALL cases[136]. Interpretation of the clinical significance of the inactivation of these tumor suppressor genes must also take into account alternative mechanisms of gene inactivation, such as aberrant methylation of *p15INK4B* promoter sequences, which has been associated with epigenetic silencing of this locus and a worse outcome in adult ALL cases[137].

Identification of recurring chromosomal deletion syndromes in human ALL indicates that other tumor suppressor loci may be involved in this disease. These syndromes, which affect the long arm of chromosome 6, the short arm of chromosome 9, or the short arm of chromosome 12, can each be found in leukemic cells from approximately 10% of patients with ALL by standard cytogenetic analysis, making them among the most frequent cytogenetic abnormalities in this disease. Functional deletion can result either from interstitial deletion of the involved chromosome arm or from derivative chromosomes that result from unbalanced chromosomal translocations. For each chromosome, the deleted regions overlap a single target region, which may contain key tumor suppressor genes, whose loss could be an important step in leukemic transformation.

Deletions of the long arm of chromosome 6 are consistently found in about 10% of cases of ALL[138]. Interstitial deletions affecting bands 6q15-q24 have been reported most frequently; translocations with breakpoints within this region are also common. Band q21 of chromosome 6 seems to be involved in each of the abnormalities, suggesting that the target gene(s) resides in this region. Deletions of chromosome 6q occur with equal frequency in pro-B, pre-B and T-cell cases.

Deletions or translocations involving the short arm of chromosome 12 are also found in about 10% of ALL cases, with most clustered around band 12p13[138]. These cases generally have a B-precursor phenotype, and the blast cells usually express CD10 and HLA-DR on the cell surface. Abnormalities of the short arm of chromosome 12 are rarely found in T-ALL cases. Translocations involving chromosome 12p13 may be balanced or unbalanced and can involve multiple different donor chromosomes.

Molecular studies, however, have revealed that the majority of translocations involving 12p13 are cryptic 12;21 translocations, resulting in the *TEL-AML1* fusion. In the cases with unbalanced translocations, DNA sequences distal to the breakpoint are lost from the affected homologue and subsequently from the leukemic cell genome, so the result is similar to that of interstitial deletion. The frequency of deletions involving the 12p13 region suggests that these lesions primarily inactivate one allele of a tumor suppressor gene in this chromosomal region. Although the *TEL* and *p27KIP1* genes may be targets of deletion in these cases, neither locus is inactivated by point mutations in childhood ALL cases with loss of heterozygosity (LOH) in 12p, possibly implicating additional tumor suppressor genes in this region, although *TEL* or *p27KIP1* haploinsuficiency could also contribute to leukemic transformation[193, 297].

Mutated *RAS* Genes

Proto-oncogenes of the *RAS* family — *HRAS*, *KRAS*, and *NRAS* — encode 21-kDa proteins that are associated with the inner surface of the cytoplasmic membrane. These proteins bind guanidine nucleotides and function as intermediates in signal transduction pathways that regulate the growth of cells. The *RAS* proto-oncogenes are activated to the status of transforming oncogenes by somatic mutations that alter the amino acids specified by codons 12, 13 or 61. Human tumor DNAs were initially found to contain activated homologues of either *HRAS* or *KRAS*[141.142], proto-oncogenes that were identified on the basis of their homology with viral oncogenes. Mutated *RAS* genes also bind guanine nucleotides, but have diminished capacity to hydrolyze GTP to GDP. Transforming properties of activated RAS proteins may result from their inability to hydrolyze GTP, which could play an important role in modulating signal transduction.

The transforming potential of human *RAS* genes activated by point mutation has been documented in experimental systems. The *RAS* oncogenes will transform NIH-3T3 murine fibroblasts in vitro, and will collaborate with other oncogenes to transform primary cultures of embryo fibroblasts[143-146]. In addition, their role in mammalian tumorigenesis has been documented in carcinogen-induced animal tumor model systems[147-149].

Activated *NRAS* genes appear to be preferentially involved in hematopoietic malignancies. They were detected in the myeloid cell lines HL-60, KG1, and Rc2A[150, 151]; in fresh leukemic cell samples from patients with AML or CML[152-154]; and in lymphoblastic leukemias with a T-cell immunophenotype[155]. In AML, *NRAS* gene mutations involving codon 13 or 61 were found in approximately 20% of cases, regardless of morphologic subtype[156, 157]. Mutation of codon 12 of the *KRAS* gene was also observed in 2 of the 37 cases studied[156]. In a study of lymphoblasts from children with ALL, 2 of 19 patients showed mutated *NRAS* genes, both involving codon 12[156]. Mutated *RAS* genes have also been documented in patients with preleukemic syndromes, indicating the potential involvement

Recent Developments

To more comprehensively assess the pathogenic contribution of the PTEN-PI3K-AKT pathway to T-cell acute lymphoblastic leukemia (T-ALL), we examined diagnostic DNA samples from children with T-ALL using array CGH and sequence analysis[157]. Alterations of *PTEN*, *PI3K* or *AKT* were identified in 47.7% of 44 cases. There was a striking clustering of *PTEN* mutations in exon 7 in 12 cases, all of which were predicted to truncate the C2 domain without disrupting the phosphatase domain of PTEN. Induction chemotherapy failed to induce remission in 3 of the 4 patients whose lymphoblasts harbored *PTEN* deletions at the time of diagnosis, compared with none of the 12 patients with mutations of *PTEN* exon 7 ($P = 0.007$), suggesting that *PTEN* deletion has more adverse therapeutic consequences than mutational disruptions that preserve the phosphatase domain. **These findings add significantly to the rationale for the development of therapies targeting the PTEN-PI3K-AKT pathway in T-ALL.**

Array comparative genomic hybridization (CGH) also identified children with T-cell acute lymphoblastic leukemia (T-ALL) at high risk of failure of induction chemotherapy using DNA copy number analysis of leukemic cells collected at diagnosis[158]. These samples represented

9 patients who failed to achieve an initial complete remission, 13 who relapsed, and 25 who became long-term event-free survivors. The findings were confirmed in an independent cohort of patients by quantitative DNA-PCR, an assay that is well-suited for clinical application. Analysis of the CGH findings in induction failure cases compared with those in which induction chemotherapy was successful identified the absence of biallelic *TCRã* locus deletion (ABD), indicative of an early thymocyte precursor prior to V(D)J recombination, as the most robust predictor of induction failure ($P = 0.0002$). This feature was also associated with markedly inferior event-free and overall survival rates ($P = 0.002$ and $P = 0.0002$, respectively). Using a rapid and inexpensive quantitative DNA-PCR assay, we validated ABD as a predictor of a poor response to induction chemotherapy in an independent series of cases.

Recent work by Coustan-Smith et al[159]. identified an early T-cell precursor (ETP) phenotype of T-ALL, defined by either a characteristic gene expression signature or by immunophenotype, which was found to predict treatment failure in pediatric T-ALL. Given that *TCRã* rearrangements occur early in normal T-cell development[160, 161], and deletions of TCR loci are significantly less frequent in ETP T-ALL[159], we suspected that our ABGD cases would demonstrate some biologic overlap with ETP T-ALL. Thus, using gene expression data available on 40 of the cases we analyzed by CGH, we found that 14 of these cases had the ETP gene expression signature by hierarchical clustering. Indeed, 7 of the 8 ABGD cases possessed this signature. We recommend that lymphoblasts from children with T-ALL should be evaluated at diagnosis for deletion within the *TCRã* locus. Patients lacking biallelic deletion, which confers a very high probability of induction failure with contemporary therapy, should be assigned to alternative therapy in the context of a prospective clinical trial.

Future Directions

Gene expression analysis of ALL samples has demonstrated that different molecular subsets of the disease have different gene expression signatures demonstrating the heterogeneity of the disease. The success of imatinib for *BCR-ABL* positive leukemia has indicated the promise of molecularly targeted therapies for leukemia and cancer in general. The discovery of activation of the NOTCH pathway in T-ALL patients has led to the opening of clinical trials with g-secretase inhibitors. Gene expression analysis has identified activation of the FLT3 pathway in MLL rearranged leukemia providing another rational target for drug therapy. Future leukemia research should focus on defining the molecular events that contribute to transformation in each subtype of lymphoid leukemia. Microarray expression analysis combined with emerging technologies such as RNAi and ChIP-on-chip analysis hold promise in reaching this goal. We imagine that in the future, molecularly targeted therapies will be available for all pathways activated in leukemia and that patients will be given compounds that target the pathways activated by the oncoproteins expressed in their leukemic blasts. Because these drugs will be more specific they should be less toxic and have fewer long-term side effects than current chemotherapeutics.

References

1. Reinherz EL, Schlossman SF. Current concepts in immunology: Regulation of the immune response—inducer and suppressor T-lymphocyte subsets in human beings. *N Engl J Med*. 1980;**303**:370-373.
2. Staal FJ, Weerkamp F, Langerak AW, *et al.* Transcriptional control of t lymphocyte differentiation. *Stem Cells*. 2001;**19**:165-179.
3. Uckun FM, Steinherz PG, Sather H, *et al.* CD2 antigen expression on leukemic cells as a predictor of event-free survival after chemotherapy for T-lineage acute lymphoblastic leukemia: a Children's Cancer Group study. *Blood*. 1996;**88**:4288-4295.
4. Czuczman MS, Dodge RK, Stewart CC, *et al.* Value of immunophenotype in intensively treated adult acute lymphoblastic leukemia: cancer and leukemia Group B study 8364. *Blood*. 1999;**93**:3931-3939.
5. Shuster JJ, Falletta JM, Pullen DJ, *et al.* Prognostic factors in childhood T-cell acute lymphoblastic leukemia: a Pediatric Oncology Group study. *Blood*. 1990; **75**:166-173.
6. Pui CH, Behm FG, Singh B, *et al.* Heterogeneity of presenting features and their relation to treatment outcome in 120 children with T-cell acute lymphoblastic leukemia. *Blood*. 1990;**75**:174-179.
7. Niehues T, Kapaun P, Harms DO, *et al.* A classification based on T cell selection-related phenotypes identifies a subgroup of childhood T-ALL with favorable outcome in the COALL studies. *Leukemia*. 1999;**13**:614-617.

8. Ferrando AA, Look AT. Clinical implications of recurring chromosomal and associated molecular abnormalities in acute lymphoblastic leukemia. *Semin Hematol*. 2000;**37**:381-395.

9. Begley CG, Aplan PD, Davey MP*, et al.* Chromosomal translocation in a human leukemic stem-cell line disrupts the T-cell antigen receptor delta-chain diversity region and results in a previously unreported fusion transcript. *Proc Natl Acad Sci U S A*. 1989;**86**:2031-2035.

10. Chen Q, Cheng JT, Tasi LH*, et al.* The tal gene undergoes chromosome translocation in T cell leukemia and potentially encodes a helix-loop-helix protein. *Embo J*. 1990;**9**:415-424.

11. Xia Y, Brown L, Yang CY*, et al.* TAL2, a helix-loop-helix gene activated by the (7;9)(q34;q32) translocation in human T-cell leukemia. *Proc Natl Acad Sci U S A*. 1991;**88**:11416-11420.

12. Mellentin JD, Smith SD, Cleary ML. lyl-1, a novel gene altered by chromosomal translocation in T cell leukemia, codes for a protein with a helix-loop-helix DNA binding motif. *Cell*. 1989;**58**:77-83.

13. Finger LR, Harvey RC, Moore RC*, et al.* A common mechanism of chromosomal translocation in T- and B-cell neoplasia. *Science*. 1986;**234**:982-985.

14. McKeithan TW, Shima EA, Le Beau MM*, et al.* Molecular cloning of the breakpoint junction of a human chromosomal 8;14 translocation involving the T-cell receptor alpha-chain gene and sequences on the 3' side of MYC. *Proc Natl Acad Sci U S A*. 1986;**83**:6636-6640.

15. Shima EA, Le Beau MM, McKeithan TW*, et al.* Gene encoding the alpha chain of the T-cell receptor is moved immediately downstream of c-myc in a chromosomal 8;14 translocation in a cell line from a human T-cell leukemia. *Proc Natl Acad Sci U S A*. 1986;**83**:3439-3443.

16. Wang J, Jani-Sait SN, Escalon EA*, et al.* The t(14;21)(q11.2;q22) chromosomal translocation associated with T-cell acute lymphoblastic leukemia activates the BHLHB1 gene. *Proc Natl Acad Sci U S A*. 2000;**97**:3497-3502.

17. McGuire EA, Hockett RD, Pollock KM*, et al.* The t(11;14)(p15;q11) in a T-cell acute lymphoblastic leukemia cell line activates multiple transcripts, including Ttg-1, a gene encoding a potential zinc finger protein. *Mol Cell Biol*. 1989;**9**:2124-2132.

18. Boehm T, Foroni L, Kaneko Y*, et al.* The rhombotin family of cysteine-rich LIM-domain oncogenes: distinct members are involved in T-cell translocations to human chromosomes 11p15 and 11p13. *Proc Natl Acad Sci U S A*. 1991;**88**:4367-4371.

19. Royer-Pokora B, Loos U, Ludwig WD. TTG-2, a new gene encoding a cysteine-rich protein with the LIM motif, is overexpressed in acute T-cell leukaemia with the t(11;14)(p13;q11). *Oncogene*. 1991;**6**:1887-1893.

20. Hatano M, Roberts CW, Minden M*, et al.* Deregulation of a homeobox gene, HOX11, by the t(10;14) in T cell leukemia. *Science*. 1991;**253**:79-82.

21. Ferrando AA, Look AT. Gene expression profiling in T-cell acute lymphoblastic leukemia. *Semin Hematol*. 2003;**40**:274-280.

22. Kennedy MA, Gonzalez-Sarmiento R, Kees UR*, et al.* HOX11, a homeobox-containing T-cell oncogene on human chromosome 10q24. *Proc Natl Acad Sci U S A*. 1991;**88**:8900-8904.

23. Bernard OA, Busson-LeConiat M, Ballerini P*, et al.* A new recurrent and specific cryptic translocation, t(5;14)(q35;q32), is associated with expression of the Hox11L2 gene in T acute lymphoblastic leukemia. *Leukemia*. 2001;**15**:1495-1504.

24. Lu M, Gong ZY, Shen WF, Ho AD. The tcl-3 proto-oncogene altered by chromosomal translocation in T-cell leukemia codes for a homeobox protein. *Embo J*. 1991;**10**:2905-2910.

25. Dube ID, Kamel-Reid S, Yuan CC*, et al.* A novel human homeobox gene lies at the chromosome 10 breakpoint in lymphoid neoplasias with chromosomal translocation t(10;14). *Blood*. 1991;**78**:2996-3003.

26. Dear TN, Sanchez-Garcia I, Rabbitts TH. The HOX11 gene encodes a DNA-binding nuclear transcription factor belonging to a distinct family of homeobox genes. *Proc Natl Acad Sci U S A*. 1993;**90**:4431-4435.

27. Brown L, Cheng JT, Chen Q*, et al.* Site-specific recombination of the tal-1 gene is a common occurrence in human T cell leukemia. *Embo J*. 1990;**9**:3343-3351.

28. Aplan PD, Lombardi DP, Kirsch IR. Structural characterization of SIL, a gene frequently disrupted in T-cell acute lymphoblastic leukemia. *Mol Cell Biol*. 1991;**11**:5462-5469.

29. Bernard O, Lecointe N, Jonveaux P*, et al.* Two site-specific deletions and t(1;14) translocation restricted to human T-cell acute leukemias disrupt the 5' part of the tal-1 gene. *Oncogene*. 1991;**6**:1477-1488.

30. Aplan PD, Lombardi DP, Reaman GH*, et al.* Involvement of the putative hematopoietic transcription factor SCL in T-cell acute lymphoblastic leukemia. *Blood*. 1992; **79**:1327-1333.

31. Breit TM, Mol EJ, Wolvers-Tettero IL*, et al.* Site-specific deletions involving the tal-1 and sil genes are restricted to cells of the T cell receptor alpha/beta lineage: T cell receptor delta gene deletion mechanism affects multiple genes. *J Exp Med*. 1993;**177**:965-977.

32. Bash RO, Hall S, Timmons CF*, et al.* Does activation of the TAL1 gene occur in a majority of patients with T-cell acute lymphoblastic leukemia? A pediatric oncology group study. *Blood*. 1995;**86**:666-676.

33. Shivdasani RA, Mayer EL, Orkin SH. Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL. *Nature*. 1995;**373**:432-434.

34. Robb L, Lyons I, Li R*, et al.* Absence of yolk sac hematopoiesis from mice with a targeted disruption of the scl gene. *Proc Natl Acad Sci U S A*. 1995;**92**:7075-7079.

35. Mikkola HK, Klintman J, Yang H*, et al.* Haematopoietic stem cells retain long-term repopulating activity and multipotency in the absence of stem-cell leukaemia SCL/tal-1 gene. *Nature*. 2003;**421**:547-551.

36. Baer R. TAL1, TAL2 and LYL1: a family of basic helix-loop-helix proteins implicated in T cell acute leukaemia. *Semin Cancer Biol*. 1993;**4**:341-347.

37. Bain G, Engel I, Robanus Maandag EC*, et al.* E2A deficiency leads to abnormalities in alphabeta T-cell development and to rapid development of T-cell lymphomas. *Mol Cell Biol*. 1997;**17**:4782-4791.

38. Yan W, Young AZ, Soares VC*, et al.* High incidence of T-cell tumors in E2A-null mice and E2A/Id1 double-knockout mice. *Mol Cell Biol*. 1997;**17**:7317-7327.

39. O'Neil J, Billa M, Oikemus S, Kelliher M. The DNA binding activity of TAL-1 is not required to induce leukemia/lymphoma in mice. *Oncogene*. 2001;**20**:3897-3905.

40. O'Neil J, Shank J, Cusson N*, et al.* TAL1/SCL induces leukemia by inhibiting the transcriptional activity of E47/HEB. *Cancer Cell*. 2004;**5**:587-596.

41. Wadman I, Li J, Bash RO*, et al.* Specific in vivo association between the bHLH and LIM proteins implicated in human T cell leukemia. *Embo J*. 1994;**13**:4831-4839.

42. Valge-Archer VE, Osada H, Warren AJ*, et al.* The LIM protein RBTN2 and the basic helix-loop-helix protein TAL1 are present in a complex in erythroid cells. *Proc Natl Acad Sci U S A*. 1994;**91**:8617-8621.

43. Osada H, Grutz GG, Axelson H*, et al.* LIM-only protein Lmo2 forms a protein complex with erythroid transcription factor GATA-1. *Leukemia*. 1997;**11 Suppl 3**:307-312.

44. Porcher C, Swat W, Rockwell K*, et al.* The T cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. *Cell*. 1996;**86**:47-57.

45. Warren AJ, Colledge WH, Carlton MB*, et al.* The oncogenic cysteine-rich LIM domain protein rbtn2 is essential for erythroid development. *Cell*. 1994;**78**:45-57.

46. McGuire EA, Rintoul CE, Sclar GM, Korsmeyer SJ. Thymic overexpression of Ttg-1 in transgenic mice results in T-cell acute lymphoblastic leukemia/lymphoma. *Mol Cell Biol*. 1992;**12**:4186-4196.

47. Fisch P, Boehm T, Lavenir I*, et al.* T-cell acute lymphoblastic lymphoma induced in transgenic mice by the RBTN1 and RBTN2 LIM-domain genes. *Oncogene*. 1992;**7**:2389-2397.

48. Larson RC, Fisch P, Larson TA*, et al.* T cell tumours of disparate phenotype in mice transgenic for Rbtn-2. *Oncogene*. 1994;**9**:3675-3681.

49. Larson RC, Osada H, Larson TA*, et al.* The oncogenic LIM protein Rbtn2 causes thymic developmental aberrations that precede malignancy in transgenic mice. *Oncogene*. 1995;**11**:853-862.

50. Neale GA, Rehg JE, Goorha RM. Ectopic expression of rhombotin-2 causes selective expansion of CD4-CD8- lymphocytes in the thymus and T-cell tumors in transgenic mice. *Blood*. 1995;**86**:3060-3071.

51. Chervinsky DS, Zhao XF, Lam DH*, et al.* Disordered T-cell development and T-cell malignancies in SCL LMO1 double-transgenic mice: parallels with E2A-deficient mice. *Mol Cell Biol*. 1999;**19**:5025-5035.

52. Larson RC, Lavenir I, Larson TA*, et al.* Protein dimerization between Lmo2 (Rbtn2) and Tal1 alters thymocyte development and potentiates T cell tumorigenesis in transgenic mice. *Embo J*. 1996;**15**:1021-1027.

53. Hacein-Bey-Abina S, von Kalle C, Schmidt M*, et al.* A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med*. 2003;**348**:255-256.

54. Hacein-Bey-Abina S, Von Kalle C, Schmidt M*, et al.* LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science*. 2003;**302**:415-419.

55. Kees UR, Heerema NA, Kumar R*, et al.* Expression of HOX11 in childhood T-lineage acute lymphoblastic leukaemia can occur in the absence of cytogenetic aberration at 10q24: a study from the Children's Cancer Group (CCG). *Leukemia*. 2003;**17**:887-893.

56. Berger R, Dastugue N, Busson M*, et al.* t(5;14)/HOX11L2-positive T-cell acute lymphoblastic leukemia. A collaborative study of the Groupe Francais de Cytogenetique Hematologique (GFCH). *Leukemia*. 2003;**17**:1851-1857.

57. Roberts CW, Shutter JR, Korsmeyer SJ. Hox11 controls the genesis of the spleen. *Nature*. 1994;**368**:747-749.

58. Dear TN, Colledge WH, Carlton MB*, et al.* The Hox11 gene is essential for cell survival during spleen development. *Development*. 1995;**121**:2909-2915.

59. Raju K, Tang S, Dube ID*, et al.* Characterization and developmental expression of Tlx-1, the murine homolog of HOX11. *Mech Dev*. 1993;**44**:51-64.

60. Roberts CW, Sonder AM, Lumsden A, Korsmeyer SJ. Development expression of Hox11 and specification of splenic cell fate. *Am J Pathol*. 1995;**146**:1089-1101.

61. Riz I, Hawley RG. G1/S transcriptional networks modulated by the HOX11/TLX1 oncogene of T-cell acute lymphoblastic leukemia. *Oncogene*. 2005;**24**:5561-5575.

62. Owens BM, Hawley TS, Spain LM*, et al.* TLX1/HOX11-mediated disruption of primary thymocyte differentiation prior to the CD4+CD8+ double-positive stage. *Br J Haematol*. 2006;**132**:216-229.

63. Ballerini P, Blaise A, Busson-Le Coniat M*, et al.* HOX11L2 expression defines a clinical subtype of pediatric T-ALL associated with poor prognosis. *Blood*. 2002;**100**:991-997.

64. Mauvieux L, Leymarie V, Helias C*, et al.* High incidence of Hox11L2 expression in children with T-ALL. *Leukemia*. 2002;**16**:2417-2422.

65. Shirasawa S, Arata A, Onimaru H*, et al.* Rnx deficiency results in congenital central hypoventilation. *Nat Genet*. 2000;**24**:287-290.

66. Soulier J, Clappier E, Cayuela JM*, et al.* HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). *Blood*. 2005;**106**:274-286.

67. Ferrando AA, Herblot S, Palomero T, *et al.* Biallelic transcriptional activation of oncogenic transcription factors in T-cell acute lymphoblastic leukemia. *Blood*. 2004;**103**:1909-1911.

68. Dreyling MH, Martinez-Climent JA, Zheng M, *et al.* The t(10;11)(p13;q14) in the U937 cell line results in the fusion of the AF10 gene and CALM, encoding a new member of the AP-3 clathrin assembly protein family. *Proc Natl Acad Sci U S A*. 1996;**93**:4804-4809.

69. Asnafi V, Radford-Weiss I, Dastugue N, *et al.* CALM-AF10 is a common fusion transcript in T-ALL and is specific to the TCRgammadelta lineage. *Blood*. 2003;**102**:1000-1006.

70. Dik WA, Brahim W, Braun C, *et al.* CALM-AF10+ T-ALL expression profiles are characterized by overexpression of HOXA and BMI1 oncogenes. *Leukemia*. 2005;**19**:1948-1957.

71. Armstrong SA, Staunton JE, Silverman LB, *et al.* MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nat Genet*. 2002;**30**:41-47.

72. Yeoh EJ, Ross ME, Shurtleff SA, *et al.* Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell*. 2002;**1**:133-143.

73. Ferrando AA, Armstrong SA, Neuberg DS, *et al.* Gene expression signatures in MLL-rearranged T-lineage and B-precursor acute leukemias: dominance of HOX dysregulation. *Blood*. 2003;**102**:262-268.

74. Ayton PM, Cleary ML. Transformation of myeloid progenitors by MLL oncoproteins is dependent on Hoxa7 and Hoxa9. *Genes Dev*. 2003;**17**:2298-2307.

75. Ferrando AA, Neuberg DS, Staunton J, *et al.* Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. *Cancer Cell*. 2002;**1**:75-87.

76. Ellisen LW, Bird J, West DC, *et al.* TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell*. 1991;**66**:649-661.

77. Pear WS, Aster JC, Scott ML, *et al.* Exclusive development of T cell neoplasms in mice transplanted with bone marrow expressing activated Notch alleles. *J Exp Med*. 1996;**183**:2283-2291.

78. Pear WS, Radtke F. Notch signaling in lymphopoiesis. *Semin Immunol*. 2003;**15**:69-79.

79. Ordentlich P, Lin A, Shen CP, *et al.* Notch inhibition of E47 supports the existence of a novel signaling pathway. *Mol Cell Biol*. 1998;**18**:2230-2239.

80. Weng AP, Ferrando AA, Lee W, *et al.* Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science*. 2004;**306**:269-271.

81. Grabher C, von Boehmer H, Look AT. Notch 1 activation in the molecular pathogenesis of T-cell acute lymphoblastic leukaemia. *Nat Rev Cancer*. 2006; **6**:347-359.

82. Meeker TC, Hardy D, Willman C, *et al.* Activation of the interleukin-3 gene by chromosome translocation in acute lymphocytic leukemia with eosinophilia. *Blood*. 1990;**76**:285-289.

83. Grimaldi JC, Meeker TC. The t(5;14) chromosomal translocation in a case of acute lymphocytic leukemia joins the interleukin-3 gene to the immunoglobulin heavy chain gene. *Blood*. 1989;**73**:2081-2085.

84. Tycko B, Smith SD, Sklar J. Chromosomal translocations joining LCK and TCRB loci in human T cell leukemia. *J Exp Med*. 1991;**174**:867-873.

85. Burnett RC, David JC, Harden AM, *et al.* The LCK gene is involved in the t(1;7)(p34;q34) in the T-cell acute lymphoblastic leukemia derived cell line, HSB-2. *Genes Chromosomes Cancer*. 1991;**3**:461-467.

86. Wright DD, Sefton BM, Kamps MP. Oncogenic activation of the Lck protein accompanies translocation of the LCK gene in the human HSB2 T-cell leukemia. *Mol Cell Biol*. 1994;**14**:2429-2437.

87. Graux C, Cools J, Melotte C, *et al.* Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. *Nat Genet*. 2004;**36**:1084-1089.

88. Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A*. 1971;**68**:820-823.

89. Nigro JM, Baker SJ, Preisinger AC, *et al.* Mutations in the p53 gene occur in diverse human tumour types. *Nature*. 1989;**342**:705-708.

90. Li FP, Fraumeni JF, Jr., Mulvihill JJ, *et al.* A cancer family syndrome in twenty-four kindreds. *Cancer Res*. 1988;**48**:5358-5362.

91. Malkin D, Li FP, Strong LC, *et al.* Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*. 1990;**250**:1233-1238.

92. Srivastava S, Zou ZQ, Pirollo K, *et al.* Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature*. 1990;**348**:747-749.

93. Frebourg T, Friend SH. Cancer risks from germline p53 mutations. *J Clin Invest*. 1992;**90**:1637-1641.

94. Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell*. 1997;**88**:323-331.

95. Matlashewski G, Lamb P, Pim D, *et al.* Isolation and characterization of a human p53 cDNA clone: expression of the human p53 gene. *Embo J*. 1984;**3**:3257-3262.

96. Zakut-Houri R, Bienz-Tadmor B, Givol D, Oren M. Human p53 cellular tumor antigen: cDNA sequence and expression in COS cells. *Embo J*. 1985;**4**:1251-1255.

97. Baker SJ, Fearon ER, Nigro JM, *et al.* Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science*. 1989;**244**:217-221.

98. Baker SJ, Markowitz S, Fearon ER, *et al.* Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science*. 1990;**249**:912-915.

99. Funk WD, Pak DT, Karas RH, *et al.* A transcriptionally

active DNA-binding site for human p53 protein complexes. *Mol Cell Biol*. 1992;**12**:2866-2871.

100. el-Deiry WS, Kern SE, Pietenpol JA*, et al.* Definition of a consensus binding site for p53. *Nat Genet*. 1992;**1**:45-49.

101. Farmer G, Bargonetti J, Zhu H*, et al.* Wild-type p53 activates transcription in vitro. *Nature*. 1992;**358**:83-86.

102. Fields S, Jang SK. Presence of a potent transcription activating sequence in the p53 protein. *Science*. 1990;**249**:1046-1049.

103. Kern SE, Kinzler KW, Bruskin A*, et al.* Identification of p53 as a sequence-specific DNA-binding protein. *Science*. 1991;**252**:1708-1711.

104. Kastan MB, Onyekwere O, Sidransky D*, et al.* Participation of p53 protein in the cellular response to DNA damage. *Cancer Res*. 1991;**51**:6304-6311.

105. Hsiao MH, Yu AL, Yeargin J*, et al.* Nonhereditary p53 mutations in T-cell acute lymphoblastic leukemia are asso ciated with the relapse phase. *Blood*. 1994;**83**:2922-2930.

106. Diccianni MB, Yu J, Hsiao M*, et al.* Clinical significance of p53 mutations in relapsed T-cell acute lymphoblastic leukemia. *Blood*. 1994;**84**:3105-3112.

107. Wada M, Bartram CR, Nakamura H*, et al.* Analysis of p53 mutations in a large series of lymphoid hematologic malignancies of childhood. *Blood*. 1993;**82**:3163-3169.

108. Marks DI, Kurz BW, Link MP*, et al.* High incidence of potential p53 inactivation in poor outcome childhood acute lymphoblastic leukemia at diagnosis. *Blood*. 1996;**87**:1155-1161.

109. Marks DI, Kurz BW, Link MP*, et al.* Altered expression of p53 and mdm-2 proteins at diagnosis is associated with early treatment failure in childhood acute lymphoblastic leukemia. *J Clin Oncol*. 1997;**15**:1158-1162.

110. Sherr CJ, Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev*. 1995;**9**:1149-1163.

111. Kamijo T, Zindy F, Roussel MF*, et al.* Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell*. 1997;**91**:649-659.

112. Quelle DE, Zindy F, Ashmun RA, Sherr CJ. Alternative reading frames of the INK4a tumor suppressor gene encode two unrelated proteins capable of inducing cell

113. Sidransky D. Two tracks but one race? Cancer genetics. *Curr Biol*. 1996;**6**:523-525.

114. Kamb A, Gruis NA, Weaver-Feldhaus J*, et al.* A cell cycle regulator potentially involved in genesis of many tumor types. *Science*. 1994;**264**:436-440.

115. Nobori T, Miura K, Wu DJ*, et al.* Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. *Nature*. 1994;**368**:753-756.

116. Zhang Y, Xiong Y, Yarbrough WG. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell*. 1998;**92**:725-734.

117. Pomerantz J, Schreiber-Agus N, Liegeois NJ*, et al.* The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. *Cell*. 1998;**92**:713-723.

118. Ashcroft M, Vousden KH. Regulation of p53 stability. *Oncogene*. 1999;**18**:7637-7643.

119. Sharpless NE, Bardeesy N, Lee KH*, et al.* Loss of p16Ink4a with retention of p19Arf predisposes mice to tumorigenesis. *Nature*. 2001;**413**:86-91.

120. Krimpenfort P, Quon KC, Mooi WJ*, et al.* Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. *Nature*. 2001;**413**:83-86.

121. Hebert J, Cayuela JM, Berkeley J, Sigaux F. Candidate tumor-suppressor genes MTS1 (p16INK4A) and MTS2 (p15INK4B) display frequent homozygous deletions in primary cells from T- but not from B-cell lineage acute lymphoblastic leukemias. *Blood*. 1994;**84**:4038-4044.

122. Quesnel B, Preudhomme C, Philippe N*, et al.* p16 gene homozygous deletions in acute lymphoblastic leukemia. *Blood*. 1995;**85**:657-663.

123. Haidar MA, Cao XB, Manshouri T*, et al.* p16INK4A and p15INK4B gene deletions in primary leukemias. *Blood*. 1995;**86**:311-315.

124. Fizzotti M, Cimino G, Pisegna S*, et al.* Detection of homozygous deletions of the cyclin-dependent kinase 4 inhibitor (p16) gene in acute lymphoblastic leukemia and association with adverse prognostic features. *Blood*. 1995;**85**:2685-2690.

125. Rasool O, Heyman M, Brandter LB*, et al.* p15ink4B and p16ink4 gene inactivation in acute lymphocytic leukemia. *Blood*. 1995;**85**:3431-3436.

126. Okuda T, Shurtleff SA, Valentine MB*, et al.* Frequent deletion of p16INK4a/MTS1 and p15INK4b/MTS2 in pediatric acute lymphoblastic leukemia. *Blood*. 1995;**85**:2321-2330.

127. Hirama T, Koeffler HP. Role of the cyclin-dependent kinase inhibitors in the development of cancer. *Blood*. 1995;**86**:841-854.

128. Iolascon A, Faienza MF, Coppola B*, et al.* Homozygous deletions of cyclin-dependent kinase inhibitor genes, p16(INK4A) and p18, in childhood T cell lineage acute lymphoblastic leukemias. *Leukemia*. 1996;**10**:255-260.

129. Nakao M, Yokota S, Kaneko H*, et al.* Alterations of CDKN2 gene structure in childhood acute lymphoblastic leukemia: mutations of CDKN2 are observed preferentially in T lineage. *Leukemia*. 1996;**10**:249-254.

130. Cayuela JM, Madani A, Sanhes L*, et al.* Multiple tumor-suppressor gene 1 inactivation is the most frequent genetic alteration in T-cell acute lymphoblastic leukemia. *Blood*. 1996;**87**:2180-2186.

131. Takeuchi S, Bartram CR, Seriu T*, et al.* Analysis of a family of cyclin-dependent kinase inhibitors: p15/MTS2/INK4B, p16/MTS1/INK4A, and p18 genes in acute lymphoblastic leukemia of childhood. *Blood*. 1995;**86**:755-760.

132. Heyman M, Rasool O, Borgonovo Brandter L*, et al.* Prognostic importance of p15INK4B and p16INK4 gene inactivation in childhood acute lymphocytic leukemia. *J Clin Oncol*. 1996;**14**:1512-1520.

133. Drexler HG. Review of alterations of the cyclin-dependent kinase inhibitor INK4 family genes p15, p16, p18 and p19 in human leukemia-lymphoma cells. *Leukemia*. 1998;**12**:845-859.

134. Kees UR, Burton PR, Lu C, Baker DL. Homozygous deletion of the p16/MTS1 gene in pediatric acute lymphoblastic leukemia is associated with unfavorable clinical outcome. *Blood*. 1997;**89**:4161-4166.

135. Yamada Y, Hatta Y, Murata K*, et al.* Deletions of p15 and/or p16 genes as a poor-prognosis factor in adult T-cell leukemia. *J Clin Oncol*. 1997;**15**:1778-1785.

136. Faderl S, Kantarjian HM, Manshouri T*, et al.* The prognostic significance of p16INK4a/p14ARF and p15INK4b deletions in adult acute lymphoblastic leukemia. *Clin Cancer Res*. 1999;**5**:1855-1861.

137. Wong IH, Ng MH, Huang DP, Lee JC. Aberrant p15 promoter methylation in adult and childhood acute leukemias of nearly all morphologic subtypes: potential prognostic implications. *Blood*. 2000;**95**:1942-1949.

138. Raimondi SC. Current status of cytogenetic research in childhood acute lymphoblastic leukemia. *Blood*. 1993;**81**:2237-2251.

139. Stegmaier K, Takeuchi S, Golub TR*, et al.* Mutational analysis of the candidate tumor suppressor genes TEL and KIP1 in childhood acute lymphoblastic leukemia. *Cancer Res*. 1996;**56**:1413-1417.

140. Cave H, Cacheux V, Raynaud S*, et al.* ETV6 is the target of chromosome 12p deletions in t(12;21) childhood acute lymphocytic leukemia. *Leukemia*. 1997;**11**:1459-1464.

141. Der CJ, Krontiris TG, Cooper GM. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. *Proc Natl Acad Sci U S A*. 1982;**79**:3637-3640.

142. Parada LF, Tabin CJ, Shih C, Weinberg RA. Human EJ bladder carcinoma oncogene is homologue of Harvey sarcoma virus ras gene. *Nature*. 1982;**297**:474-478.

143. Land H, Parada LF, Weinberg RA. Cellular oncogenes and multistep carcinogenesis. *Science*. 1983;**222**:771-778.

144. Land H, Parada LF, Weinberg RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature*. 1983;**304**:596-602.

145. Eliyahu D, Raz A, Gruss P*, et al.* Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. *Nature*. 1984;**312**:646-649.

146. Parada LF, Land H, Weinberg RA*, et al.* Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. *Nature*. 1984;**312**:649-651.

147. Balmain A, Pragnell IB. Mouse skin carcinomas induced in vivo by chemical carcinogens have a transforming Harvey-ras oncogene. *Nature*. 1983;**303**:72-74.

148. Sukumar S, Notario V, Martin-Zanca D, Barbacid M. Induction of mammary carcinomas in rats by nitroso-methylurea involves malignant activation of H-ras-1 locus by single point mutations. *Nature*. 1983;**306**:658-661.

149. Guerrero I, Calzada P, Mayer A, Pellicer A. A molecular approach to leukemogenesis: mouse lymphomas contain an activated c-ras oncogene. *Proc Natl Acad Sci U S A*. 1984;**81**:202-205.

150. Murray MJ, Cunningham JM, Parada LF*, et al.* The HL-60 transforming sequence: a ras oncogene coexisting with altered myc genes in hematopoietic tumors. *Cell*. 1983;**33**:749-757.

151. Bos JL, Toksoz D, Marshall CJ*, et al.* Amino-acid substitutions at codon 13 of the N-ras oncogene in human acute myeloid leukaemia. *Nature*. 1985; **315**:726-730.

152. Gambke C, Signer E, Moroni C. Activation of N-ras gene in bone marrow cells from a patient with acute myeloblastic leukaemia. *Nature*. 1984;**307**:476-478.

153. Hirai H, Tanaka S, Azuma M*, et al.* Transforming genes in human leukemia cells. *Blood*. 1985;**66**:1371-1378.

154. Souyri M, Fleissner E. Identification by transfection of transforming sequences in DNA of human T-cell leukemias. *Proc Natl Acad Sci U S A*. 1983;**80**:6676-6679.

155. Bos JL, Verlaan-de Vries M, van der Eb AJ*, et al.* Mutations in N-ras predominate in acute myeloid leukemia. *Blood*. 1987;**69**:1237-1241.

156. Rodenhuis S, Bos JL, Slater RM*, et al.* Absence of oncogene amplifications and occasional activation of N-ras in lymphoblastic leukemia of childhood. *Blood*. 1986;**67**:1698-1704.

157. Gutierrez A, Sanda T, Grebliunaite R, *et al.* High frequency of PTEN, P13K, and AKT abnormalities in T cell acute lymphoblastic leukemia. *Blood.* 2009; **114:**647-650.

158. Gutierrez A, Dahlberg SE, Neuberg DS, *et al.* Absence of Biallelic *TCR*ã Deletion Predicts Early Treatment Failure in Pediatric T-Cell Acute Lymphoblastic Leukemia. *J. Clin. Oncol.* 2010 July 19 (Epub ahead of print).

159. Coustan-Smith E, Mullighan CG, Onciu M, et al: Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. *Lancet Oncol* 2009;**10:**147-156.

160. Blom B, Verschuren MC, Heemskerk MH, *et al:* TCR gene rearrangements and expression of the pre-T cell receptor complex during human T-cell differentiation. *Blood* **1999; 93:**3033-3043.

161. Dik WA, Pike-Overzet K, Weerkam, et al: New insights on human T cell development by quantitative T cell receptor gene rearrangement studies and gene expression profiling. *J. Exp. Med.* 2005;**201**:1715-1723.

Relapsed Acute Lymphoblastic Leukemia - Overview

Günter Henze

1 Introduction

Relapses of childhood acute lymphoblastic leukemia (ALL) have became less frequent because with currently used front-line treatment event-free survival rates have reached over 80%. Nevertheless, the absolute number of relapses is still substantial because ALL is the most frequent type of childhood malignancies. Problems to manage ALL relapse are the resistance of the leukemic cells and the reduced tolerance of patients to a second round of treatment after having already received one of the currently applied intensive frontline therapies. Therefore, the treatment has to be carefully planned and adapted to the patients' individual needs.

2 Diagnosis of Relapse

Relapse of ALL is defined as the reappearance of leukemic cells in any anatomic compartment following CR. This must be proven beyond any doubts. Therefore, the diagnostic work-up has to be done following the same rules as in initial ALL, including a careful physical examination, cytological, cytogenetic and molecular genetic investigation and immune phenotyping of bone marrow (BM), biopsies if appropriate (e.g., the testicles, lymph nodes or any other organs or tissues), and investigation of the cerebrospinal fluid (CSF).

Isolated BM relapse is defined as the presence of at least 25% leukemic cells in BM without evidence of extramedullary leukemia. Isolated extramedullary (EM) relapses are defined as histologically proven leukemia in one or more EM organs and less than 5% leukemia cells in BM; in combined EM relapses at least 5% leukemic blasts have to be present in the BM.

Typical extramedullary sites of relapse are the central nervous system (CNS) and the testicles. The diagnosis of a CNS relapse requires the presence of at least 5 white blood cells (WBC) per microliter with blast cell morphology (cytospin preparation). Rarely MRI may be required to detect CNS leukemia.

Typical for a testicular relapse is unilateral or bilateral painless testicular swelling. Biopsies should always be performed of both testicles for histological investigation. Less frequently, leukemia may recur at other extramedullary sites (skin, bone and muscle, abdominal organs, or the eye).

Immunophenotyping and cytogenetic analyses of cells should follow the same guidelines as applied at the first manifestation of ALL. Likewise, molecular genetic investigations should be performed to detect clone-specific rearrangements of T-cell receptor and immunoglobulin genes. These can be used to quantitatively assess the response to therapy, to monitor minimal residual disease and to plan appropriate therapy, accordingly.

3 Prognostic Factors

The most relevant prognostic factor is the duration of first remission. In the BFM Study Group, relapses are defined as very early if they occur within 18 months after initial diagnosis, early if the occur beyond 18 months after initial diagnosis and up to 6 months after the cessation of frontline treatment; all others (beyond 6 months after the cessation of frontline treatment) are termed late relapses. Kaplan-Meier plots for event-free survival of 910 patients treated between 1983 and 1997 in trials ALL-REZ BFM 83, 85, 87, 90, and 95 based on these definitions are shown in ure 1. These definitions are not uniformly but similarly used among different study groups. Different definitions must be considered when comparing the results of clinical trials.

In addition to duration of first remission, site of relapse is a relevant predictor of prognosis. Children with extramedullary relapses have a better outcome compared to those with an isolated BM relapse. A reason might be that part of extramedullary relapses may have taken their origin from leukemic cells having survived in an extra-compartmental sanctuary where they were less intensively exposed to chemotherapy and thereby less likely to develop resistance against chemotherapeutic drugs. As a rule, EM relapses require systemic chemotherapy because leukemia is never really "local" and in addition adequate local treatment.

Interestingly, children with a combined BM relapse have been reported to have a better prognosis compared to that of children with an isolated BM relapse. A possible explanation may be that in combined relapses the BM blasts originate from extramedullary sites that have reseeded the marrow and have similar properties as cells in isolated EM relapses.

Another significant adverse prognostic factor in the experience of the ALL-REZ BFM study group is T-cell immunology. Relapses of T-cell ALL tend to occur early, and the rate of nonresponse to salvage treatment is high. Second remissions are of short duration. The stage of maturation of the T-cell and B-cell precursor lineages, as well as the presence of myeloid markers, has no additional prognostic relevance in relapsed ALL.

Based on time to and site of relapse, as well as immunophenotype, patients can be divided into standard-(SR), intermediate- (IR) and high-risk (HR) groups (Table 1). Kaplan-Meier plots according to this stratification are shown in figure 2. The usefulness of this prognostic classification could be confirmed by several other groups. In recent years it could be shown that further sub-classification of patients within the IR group is possible by measuring early response to therapy with MRD techniques. Patients with MRD $< 10^{-3}$ on day 36 of therapy have been shown to have a favorable outcome with chemotherapy whereas less well responding patients need treatment intensification with allogeneic SCT.

Like in front-line ALL, the detection of a Philadelphia chromosome, the translocation t(9;22), or its molecular equivalent, the fusion transcript BCR-ABL, predicts an unfavorable prognosis. Relapsing patients with BCR-ABL positive ALL have become rare, however, because the translocation is mostly detected at first diagnosis and treatment adjusted, accordingly.

Equally, like in front-line ALL, the presence of the cryptic translocation t(12;21)(p13;q22) with the resulting fusion transcript TEL-AML1 or ETV6/RUNX1 at relapse predicts a rather favorable outcome. At first diagnosis, about 25% of patients have TEL-AML1+ ALL. These patients were reported to have less and if at all rather late occurring relapses. At relapse, TEL-AML1 positive ALL represents still about 20%, and the affected children have a better prognosis compared to TEL-AML1 negative patients. In face of recent reports such late TEL-AML1 positive "relapses" may at least in part be secondary leukemias. TEL-AML1 has been detected in 1% or 2% of normal cord blood samples and is thought to be a pre-leukemic genetic alteration requiring a second event in order to progress to true leukemia. Therefore, a pre-leukemic TEL-AML1-positive clone may persist after antileukemic treatment and, after another "second" hit, may emerge as an apparent relapse.

Table 1. Stratification groups S1 – S4 of trial ALL-REZ BFM 96 defined by the prognostic factors time, site, and immunophenotype of relapse

	B-cell precursor			(Pre-)T-cell		
Time	Extra-medullary	Combined BM	Isolated BM	Extra-medullary	Combined BM	Isolated BM
Very early	IR	HR	HR	IR	HR	HR
Early	IR	IR	HR	IR	HR	HR
Late	SR	IR	IR	SR	HR	HR

Abbreviations: BM, bone marrow; IR, intermediate risk; SR, standard risk; HR, high risk.

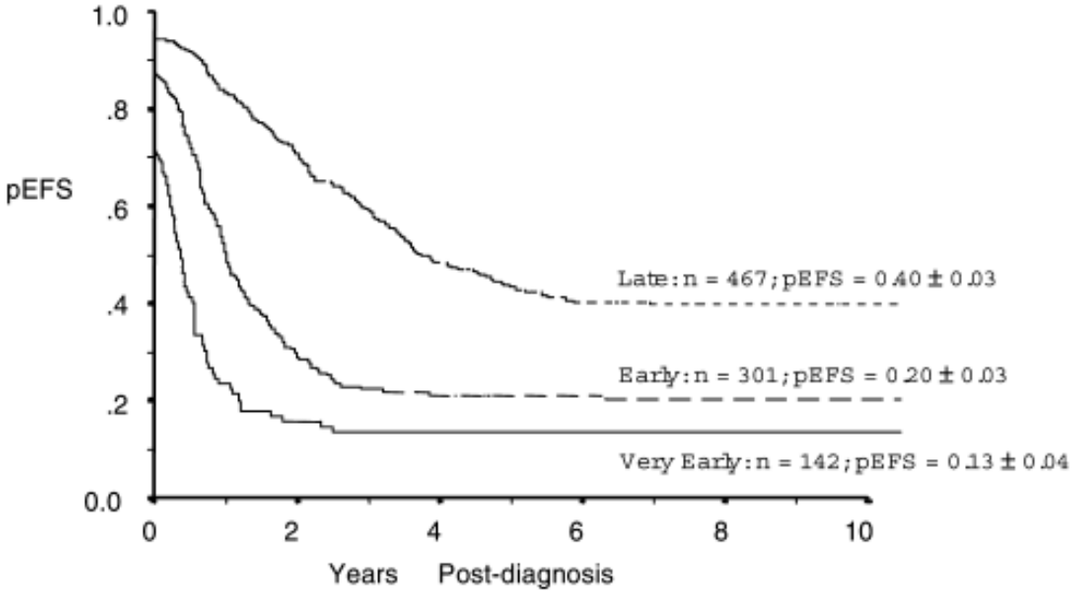

Figure 1.

Event-free survival probability (pEFS) for children with ALL in relapse according to time to relapse (SCT censored; P<0.001 by log-rank test). The patients were treated in trials ALL-REZ BFM 83-95.

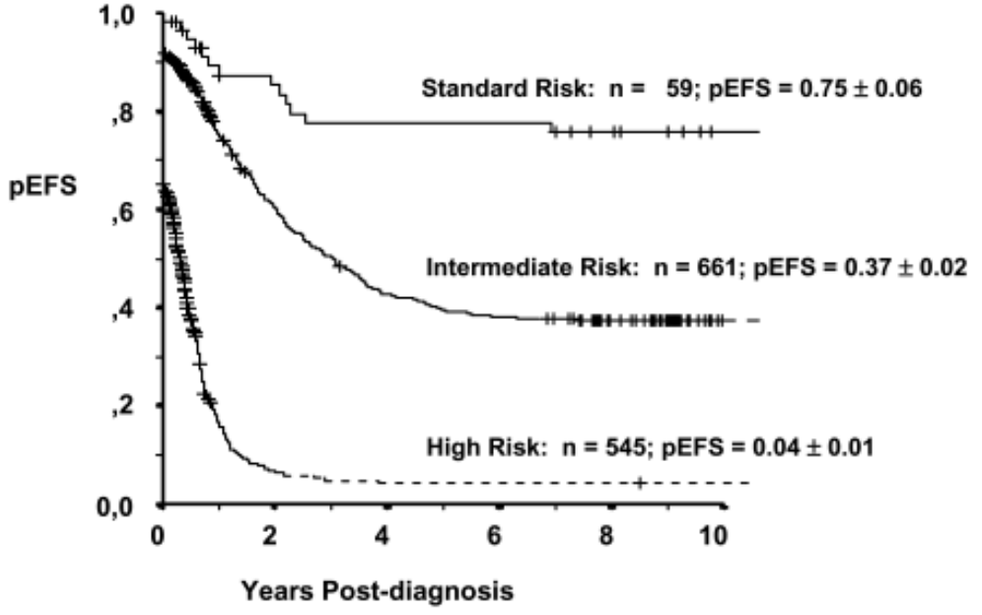

Figure 2.

Event-free survival probability (pEFS) for children with ALL in relapse according to risk-group assignment (SCT censored; P<0.001 by log-rank test). The patients were treated in trials ALL-REZ BFM 83-95

4 Treatment of relapse

Aims of treatment are to achieve a 2nd remission and to maintain the CR with either chemotherapy or stem cell transplantation. Remission rates are lower than in front-line ALL due to resistance of cells, and for the same reason subsequent relapse rates are higher resulting in less favorable overall outcome. Unlike in front-line ALL, comparison of treatment results from different study groups is difficult because of various confounders. Table 2 shows an overview of some representative treatment results reported from different study groups.

Remission rates for children with BM relapses depend on time to relapse, site, immune subtype, size of the patient cohort and also the type and intensity of front-line therapy. Reinduction therapy in BFM relapse protocols consists of dexamethasone, vincristine, L-asparaginase, intermediate dose MTX, and high-dose cytarabine, followed by two alternating 5- to 8-day multidrug courses, R1 and R2, containing glucocorticoids, thiopurines, vinca alkaloids, epipodophyllotoxins, oxazaphosphorines, intermediate- or high-dose MTX, daunorubicin, cytarabine and intrathecal MTX.

Remission rates are rather low (60-80%) in children with early BM relapse and clearly higher in late BM and EM relapses (90% or more). In the BFM trials, evidence was found for better remission rates and even better EFS rates with higher initial dose intensity of the reinduction therapy.

Table 2. Treatment results of different study groups for children with ALL in relapse

Group	Author	Protocol	Site and time of relapse	Number of patients	EFS/DFS rate
ALL-REZ BFM	Tallen at al, 2010	ALL-REZ BFM 90	BM, early	126	EFS = 17%
			BM, late	183	EFS = 43 %
	Henze et al., 1997	ALL-REZ BFM 83/ 85/87/90	CNS, isolated	73	EFS = 42 %
	Wolfrom et al., 1997	ALL-REZ BFM 83/ 85/87/90	Testis, isolated	59	EFS = 53 %
CCG	Gaynon et al., 1998	CCG 100 series 1983 – 89	BM, early[b]	267	DFS = 5 – 9 %
			BM, intermediate[b]	220	DFS = 10 – 11 %
			BM, late[b]	275	DFS = 33 – 48 %
			CNS, isolated	220	DFS = 37 %
			Testis, isolated	112	DFS = 64 %
MRC/ UKALL	Wheeler et al., 1998	UKALL X, 1985 - 93	BM, early[c]	106	DFS = 0 – 11 %
			BM, intermediate[c]	57	DFS = 14 – 40 %
			BM, late[c]	169	DFS = 33 – 50 %
	Lawson et al., 2000[98]	MRC/UKALL, R1 1991-99	BM, early[c]	29	DFS = 0 – 5 %
			BM, intermediate[c]	39	DFS = 25 – 41 %
			BM, late[b]	119	DFS = 51 – 81 %
			CNS, isolated	26	DFS = 58 %
	Grundy et al., 1997	UKALL, 1972-87	Testis, isolated	33	EFS = 59 %
POG	Buchanan et al., 2000	POG 8303, 1982-87	BM, early[a]	297	DFS = 8 %
	Sadowitz et al., 1993	POG 8304, 1983-89	BM, late[a]	105	EFS = 37 %
	Ritchey et al., 1999	POG 9061, 1990-93	CNS, isolated	83	EFS = 70 %
	Wofford et al., 1992	POG 8304, 1983-89	Testis, isolated	80	EFS = 53 – 84 %

Abbreviations: BM, bone marrow; EFS, event-free survival; DFS, disease-free survival; CNS, central nervous system; BFM, Berlin/Frankfurt/Münster; CCG, Children´s Cancer Study Group; MRC, Medical Research Council; POG, Pediatric Oncology Study Group; UKALL, United Kingdom ALL Study Group.

Definitions of time to relapse:

Definitions	Early	Intermediate	Late
a	< 6	None	> 6 months after end of frontline therapy
b	<18	18 - 36	> 36 months after initial diagnosis
c	< 24	24 - 36	> 36 months after initial diagnosis

4.1 Postremission chemotherapy

Variable combinations and schedules have been employed as postremission chemotherapy, either in form of intensive short-term courses or as prolonged continuous therapy. Until now, there is no clear evidence of superiority of either approach. Children suffering from very early, early BM or T-cell relapses require allogeneic stem cell transplantations (SCT) to achieve durable second remissions and cannot be salvaged with chemotherapy alone. In contrast, children with late BM relapses and rapid early response to induction therapy as assessed by MRD measurements can successfully be treated with chemotherapy, and SCT is only required for those with delayed and/or insufficient response. Maintenance chemotherapy appears to be indispensable in patients who are not undergoing SCT. As in newly diagnosed ALL, effective protection of the CNS is necessary, either with radiation therapy as in the BFM trials or with prolonged (triple?) intrathecal therapy.

4.1.1 Extramedullary involvement

In patients with CNS involvement, cranial irradiation is deemed necessary and should be administered at the end of intensive therapy. Whether craniospinal irradiation is superior to cranial irradiation remains unclear. Likewise, there is no clear evidence that SCT is beneficial for the treatment of CNS relapse.

The testicles are the third most frequent site of relapse. As testicular relapses tend to occur late the outcome is rather favorable. Mostly, bilateral irradiation at a dose of at least 20 Gy is recommended. We feel, however, that removal of clinically involved testicles should be preferred because 1. following irradiation subsequent relapses have been reported and 2. the function of the testes, in particular fertility, is not preserved. In overt unilateral relapse subclinical involvement of the contralateral testis has to be excluded by biopsy and “preventive” radiation therapy be administered at a dose of 15 Gy which allows still spontaneous puberty.

4.2 Stem cell transplantation (SCT)

Allogeneic SCT is required and indicated for children with very early, early and systemic T-cell ALL relapses as well as for children with late BM relapses and delayed response to induction therapy. In addition to the conditioning regimen(s) - preferably high dose VP16 plus total body irradiation - allogeneic SCT provides an antileukemic effect by a reaction of donor immune cells against residual leukemic cells, the graft-versus-leukemia (GvL) effect, in the recipient. The GvL effect is, however, mostly associated with graft-versus-host disease (GvHD), a nonspecific reaction against cells of the recipient and responsible for the higher treatment-related morbidity and mortality associated with allogeneic SCT.

Allogeneic SCT is superior to chemotherapy in preventing subsequent relapses but is associated with higher acute and long-term toxicity. It appears that a higher residual leukemic cell burden (MRD) prior to SCT is followed by a higher rate of subsequent relapses. Manipulation of the graft and/or intervention measures may be useful to reduce or predict subsequent relapses at an early stage. There is, however, no proof that these procedures have indeed a major impact on outcome.

In recent years, SCT from matched unrelated donors (MUD) have increasingly been performed with better success due to more precise (molecular) HLA typing and matching. Currently, toxicity and results with MUD-SCT have become comparable to matched sibling donor transplants.

If no donor can be found SCT from mismatched or even haploidentical donors, e. g. parents, may be performed in patients with a particularly unfavorable prognosis. Autologous transplants have largely been abandoned in ALL.

4.3 Experimental approaches and new drugs

In recent years, a number of new agents, in part “targeted” drugs have become available or are currently being investigated. Amongst them are some novel nucleoside analogues, tyrosine kinase-, NFkappaB-, m-TOR-, and FLT3 – inhibitors as well as leukemia directed monoclonal antibodies. Only few have been already licensed for use in children.

5 Conclusion

With currently available therapy modalities EFS rates of about 45% and overall survival rates of over 50% can be achieved. Thus, as in front-line

therapy major success has also been made in the treatment of relapsed ALL, and the overall survival of childhood ALL has currently reached more than 90%. Careful diagnostic work-up and treatment planning is required in order to obtain the best result for each individual patient, and even after a relapse has occurred the prognosis should not be seen as hopeless.

References

1. Mitchell C, Richards S, Harrison CJ, Eden T. Long-term follow-up of the United Kingdom medical research council protocols for childhood acute lymphoblastic leukaemia, 1980-2001. Leukemia 2010;24(2):406-18.
2. Moricke A, Zimmermann M, Reiter A, Henze G, Schrauder A, Gadner H, et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia 2009;24(2):265-84.
3. Gaynon PS. Childhood acute lymphoblastic leukaemia and relapse. Br J Haematol. 2005;131(5):579-87.
4. Henze G. Chemotherapy for relapsed childhood acute lymphoblastic leukemia. Int J Pediatr Hematol Oncol 1997;5(2-4):199-213.
5. Eckert C, Biondi A, Seeger K, Cazzaniga G, Hartmann R, Beyermann B, et al. Prognostic value of minimal residual disease in relapsed childhood acute lymphoblastic leukaemia. Lancet 2001;358(9289):1239-41.
6. Hagedorn N, Acquaviva C, Fronkova E, von Stackelberg A, Barth A, zur Stadt U, et al. Submicroscopic bone marrow involvement in isolated extramedullary relapses in childhood acute lymphoblastic leukemia: a more precise definition of "isolated" and its possible clinical implications, a collaborative study of the Resistant Disease Committee of the International BFM study group. Blood. 2007;110(12):4022-9. Epub 2007 Aug 24.
7. Bruggemann M, Schrauder A, Raff T, Pfeifer H, Dworzak M, Ottmann OG, et al. Standardized MRD quantification in European ALL trials: Proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18-20 September 2008. Leukemia 2009.
8. Tallen G, Ratei R, Mann G, Kaspers GJ, Niggli F, Karachunsky A, et al. Long-Term Outcome in Children With Relapsed Acute Lymphoblastic Leukemia After Time-Point and Site-of-Relapse Stratification and Intensified Short-Course Multidrug Chemotherapy: Results of Trial ALL-REZ BFM 90. J Clin Oncol 2010;accepted for publication.
9. Schultz KR, Bowman WP, Aledo A, Slayton WB, Sather H, Devidas M, et al. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. J Clin Oncol 2009;27(31):5175-81.
10. Zuna J, Ford AM, Peham M, Patel N, Saha V, Eckert C, et al. TEL deletion analysis supports a novel view of relapse in childhood acute lymphoblastic leukemia. Clin Cancer Res 2004;10(16):5355-60.
11. Henze G, Charite University Berlin, Germany. ALL-REZ BFM 2002: Multi-Center Study for Children With Relapsed Acute Lymphoblastic Leukemia. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [cited 2009 Jul 18]. available from: http://clinicaltrials.gov/ct2/show/NCT00114348. NLM identifier: NCT00114348.; 2005.
12. Saha V, Manchester, England, UK. ALLR3: An International Collaborative Trial for Relapsed and Refractory Acute Lymphoblastic Leukaemia (ALL). ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [cited 2009 Sep 29]. available from: http://clinicaltrials.gov/ct2/show/NCT00967057. NLM identifier: NCT00967057. 2002.
13. Raetz EA, Borowitz MJ, Devidas M, Linda SB, Hunger SP, Winick NJ, et al. Reinduction platform for children with first marrow relapse of acute lymphoblastic Leukemia: A Children's Oncology Group Study [corrected]. J Clin Oncol 2008;26(24):3971-8.
14. Barredo JC, Devidas M, Lauer SJ, Billett A, Marymont M, Pullen J, et al. Isolated CNS relapse of acute lymphoblastic leukemia treated with intensive systemic chemotherapy and delayed CNS radiation: a pediatric oncology group study. J Clin Oncol 2006;24(19):3142-9.
15. Bader P, Kreyenberg H, Henze GH, Eckert C, Reising M, Willasch A, et al. Prognostic value of minimal residual disease quantification before allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. J Clin Oncol 2009;27(3):377-84.
16. Schrauder A, von Stackelberg A, Schrappe M, Cornish J, Peters C. Allogeneic hematopoietic SCT in children with ALL: current concepts of ongoing prospective SCT trials. Bone Marrow Transplant 2008;41 Suppl 2:S71-4.
17. Pui CH, Jeha S. New therapeutic strategies for the treatment of acute lymphoblastic leukaemia. Nat Rev Drug Discov 2007;6(2):149-65.
18. Topp M, Goekbuget N, Kufer P, Zugmaier G, Degenhard E, Neumann S, et al. Treatment with Anti-CD19 BiTE Antibody Blinatumomab (MT103 / MEDI-538) Is Able to Eliminate Minimal Residual Disease (MRD) in Patients with B-Precursor Acute Lymphoblastic Leukemia (ALL): First Results of An Ongoing Phase II Study. ASH Annual Meeting Abstracts 2008;112(11):1926-.

SECTION B

Cancer Survival Need not be Determined by Income: Lessons from Developing Countries and Focusing on Children[1]

Felicia Knaul

Though often not perceived as such, cancer is a leading cause of death and disability in the developing world. Of the 7 million cancer deaths in the world today, approximately 70% occur in developing countries.[2] By 2030, low and middle income countries will bear the brunt of an estimated 27 million new cancer cases and 17 million cancer deaths. Cancer is a sorely neglected health problem and a significant cause of premature death in resource-poor settings.[2,3,4,5,6]

Case fatality for the cancers that can be treated or prevented is much higher in the developing world – a result of grave inequity in the opportunity to survive the disease. In the case of pediatric cancers – one of main causes of childhood death particularly in middle-income countries — the differentials are staggering. Data from Globocan show the ratio of deaths to incident cases in 2002 for childhood leukemia was an estimated 78% in low-income countries, 75% in low-middle income countries and 57% in high-middle income developing countries. In the developed world, it was 25%.[2]

These differentials denote the scope for action as well as the extent of inaction. Less than 5% of global resources for cancer are spent in the developing world, yet these countries account for almost 80% of disability adjusted years of life lost to cancer globally - resulting in a huge ´5/80 cancer disequilibrium´.[1,2,3]

Meanwhile, the world has witnessed unprecedented success in mobilizing resources for global health. New global and regional mechanisms have innovated financing and procurement schemes to guarantee access to much needed vaccines and medications. As a result, millions of lives have been saved. Lessons from these initiatives – particularly from AIDS — can help meet the challenge of cancer.

The commonly held assumption that cancers will remain untreated in poor countries has gone largely unchallenged in public health. Skepticism about scaling up access to treatment – as well as early detection and even palliation — in poor countries abounds.

It is time to challenge this misconception. An agenda for action on treatment should catalyze opportunities to provide expanded cancer care and control appropriate to the health systems of developing countries and accessible to poor patients. This agenda must include developing and implementing innovative health care delivery options to support rapid scale-up and by applying a diagonal approach in which resources for particular diseases are deployed in ways that strengthen entire health systems.[11]

There are important examples even in the poorest nations of successful programs to treat cancer. Several of the most inspiring examples come from children's cancers such as the St Jude Children's Research Hospital twinning programs and Mexico's *Seguro Popular*.[7],[8] These will be further discussed in the conference presentation.

Global Task Force on Expanded Cancer Care and Control in the Developing World (GTF.CCC)

The mandate of the Global Task Force on Expanded Access to Cancer Care and Control in Developing Countries (GTF.CCC) is to design, implement and evaluate innovative strategies for expanding access to cancer prevention, detection and care. The initiative focuses on the creation of global facilities and strategies for the financing and procurement of affordable, essential cancer drugs, vaccines and services for prevention, diagnosis, treatment, survivorship and palliation. Through local partners, the GTF.CCC supports implementation of innovative

service delivery models that provide evidence for scaling up access to cancer care and control, and strengthening health systems in developing countries.

The Task Force brings together leaders from the cancer and global health communities in a 22-member body that includes a Secretariat. The GTF.CCC is co-Chaired by Julio Frenk, Dean of the Harvard School of Public Health and Lawrence Shulman, Chief Medical Officer and Vice President for Medical Affairs at the Dana Farber Cancer Institute. Her Royal Highness Princess Dina Mired of the Hashemite Kingdom of Jordan and Lance Armstrong serve as Honorary co-Presidents. The Harvard Global Equity Initiative, under the direction of Felicia Knaul, serves as the Technical Secretariat for the Task Force. Work to convene the Task Force began in November of 2009 under the leadership of the Harvard Medical School, the Harvard School of Public Health, the Dana-Farber Cancer Institute and the Harvard Global Equity Initiative.

In addition to strongly supporting efforts to prevent the cancers of tomorrow by reducing cancer risk factors - and especially tobacco, the GTF.CCC calls for immediate action around treatment. To push forward this agenda, the GTF.CCC is applying the knowledge and ability of its members, combining expertise in global health and cancer, to:

- Raise global awareness of the impact of cancer on developing countries at the global, regional and national levels through an evidence-based call-to-action.
- Define the packages of essential services and treatments needed to provide care in low-resource settings for cancers which can be cured or palliated with currently available therapies.
- Reduce human suffering from all cancers by promoting universal access to pain control and palliation and increase access to the best treatment for cancer through the procurement of affordable drugs and services in line with the packages of essential package.
- Develop and evaluate innovative service delivery models that harness existing human, physical and technological resources in different economic and health system settings and share the lessons and evidence locally, regionally and globally.
- Expand the leadership, stewardship and evidence base for implementing the most efficient approaches to cancer care and control in developing countries.

The GTF.CCC is predicated on the conviction that solutions to barriers exist and that the reasons for rapidly scaling-up cancer treatment are compelling enough to merit an invigorated global response to cancer. The GTF.CCC will focus on areas that have largely been neglected, working from the perspective of health system strengthening. Specifically, GTF.CCC focuses on developing and implementing pathways to expand coverage of: 1) existing vaccines, 2) early detection and treatment of cancers where cure and major improvements in life expectancy are likely, and 3) palliation to reduce human suffering.

Proposed strategies are based on a diagonal approach designed to strengthen health systems for cancer care and control, as well as for treating other diseases and serving the population at large. This approach argues that expanding cancer treatment, rather than taking resources away from other diseases, can improve the capacity of developing countries health systems.[9] [10] [11] Strong health systems are required for effectively treating cancers, and at the same time expanding cancer care and control can strengthen health systems. An example is pain control – a right that is crucial for cancer palliation and for many other patient needs – but is often unavailable despite being low-cost.

A key contribution of the Task Force is a White Paper being produced in 2010. The content of the White paper follows from the mandate of the Task Force and provides the building blocks of a strategy for expanded cancer care and control strategies in the developing world.

Innovation Initiatives

The Task Force calls for large-scale demonstration programs to define and build new infrastructure, train health professionals and paraprofessionals, harness the opportunities of technology and especially telecommunications

to leapfrog over many of the on-site limitations in resources. Carefully designed evaluation and monitoring of these experiences will enable identification of the most effective measures to alleviate cancer burdens in different parts of the developing world and expand the volume of health services, as well as providing lessons for all health systems including the developed world.

The GTF.CCC is contributing to implementing this recommendation. The focus on developing strategies at the country level to increase access to all facets of cancer care and control has spurred partnerships in five countries of differing levels of income. This work includes developing, designing, implementing and evaluating innovations in delivery in the areas of task shifting, infrastructure shifting and use of telecommunications.

Through the following partnerships with locally entrenched and independently sustainable programs – called Innovation Initiatives - GTF.CCC is collaborating to identify strategies and lessons for expanding access to cancer care and control.

1) *Successful treatment in extremely resource-poor settings: Malawi, Rwanda, Haiti*

One commonly cited barrier to cancer treatment in resource-poor settings is the absence of specialists and specialty centers. An international partnership between Partners in Health and the Dana-Farber Cancer Institute, Harvard Medical School, and Brigham and Women's Hospital working in rural Malawi, Rwanda and Haiti is proving that this barrier can be surmounted even in the poorest settings.

PIH operates health centers and hospitals in rural districts in partnership with national Ministries of Health. With no oncologists available, care is administered by local physicians and nurse teams. Through permanent e-link up to, and training from, the Boston-based facilities of the Dana Farber Cancer Institute and the Brigham and Women´s Hospital, these centers and hospitals are delivering chemotherapy to patients presenting with a variety of treatable malignancies. To further expand access to care, the projects seek to broaden the availability of essential drugs.

2) *Expanding access to treatment through a national center of excellence: Jordan*

The King Hussein Cancer Center (KHCC) is the only Joint Commission-certified cancer center in a developing country and offers high-quality cancer treatment to patients with no means of covering the costs of their treatment. Beyond service provision, KHCC serves other key functions in 1) providing proof of concept to drive policy around the provision of high-quality care to all population groups; 2) serving as a model and catalyst to scale-up delivery, 3) playing a pivotal role in promoting a dialogue across sectors to steer the course for policy change, and 4) catalyzing key instruments such as the national cancer registry and guidelines for expanded access in a resource-poor setting.

Still, national shortages of human resources and infrastructure are evident in the face of Jordan's projected increase in cancer and the growing demand for services across the country. By strengthening the linkages between KHCC and less- comprehensive cancer care providers based outside of Amman, and implementing innovative communication and institutional exchange strategies among institutions, this project will increase access to quality care across Jordan.

3) *Including cancer treatment in national health insurance programs and harnessing the primary and secondary levels of care to increase access to breast cancer detection, treatment and survivorship: Mexico*

A key aspect of scale-up of cancer treatment and one that is part of strengthening health systems is developing explicit entitlements to health care and financial protection. In Mexico, recognition of the growing burden and the opportunity to treat has been transformed into action as part of ongoing efforts to strengthen the health system. Through the Popular Health Insurance (PHI) the range of entitlement to cancer treatment has been steadily expanded. Comprehensive treatment regimes for cervical, breast and a range of childhood and adolescent cancers are covered for all Mexicans.

Focusing on breast cancer, the projects that constitute this Innovation Initiative focus on harnessing the primary and secondary levels of

care to: increase the coverage of early detection by training basic health care workers; improve ease of access and hence uptake of certain treatment services (eg chemotherapies) and survivorship care at local hospitals with links to specialty level centers; and, generate effective options for measuring success through cancer registries.

The projects are being piloted in the states of Morelos, Jalisco and Nuevo Leon with support from Mexico´s Commission for Social Protection in Health (Seguro Popular), and as a joint learning initiative of the Ministries of Health of the states of Morelos, Jalisco and Nuevo Leon; the Mexican Health Foundation, the program Cáncer de mama: Tómatelo a pecho; the National Cancer Institute of Mexico; and the National Institute of Public Health of Mexico.

References

1 Paul Farmer, Julio Frenk, Felicia M Knaul, Lawrence N Shulman, George Alleyne, Lance Armstrong, Rifat Atun, Douglas Blayney, Lincoln Chen, Richard Feachem, Mary Gospodarowicz, Julie Gralow, Sanjay Gupta, Ana Langer, Julian Lob-Levyt, Claire Neal, Anthony MBewu, Dina Mired, Peter Piot, K Srinath Reddy, Jeffrey D Sachs, Mahmoud Sarhan, John R Seffrin (Forthcoming). Expansion of cancer care and control in low-income and middle-income countries: a call to action. Lancet.

2 Beaulieu N, Bloom D, Bloom R, Stein R. Breakaway: The global burden of cancer - challenges and opportunities. The Economist Intelligence Unit. 2009.

3 Cancer control opportunities in low- and middle-income countries. Washington, DC: Institute of Medicine of the National Academies, National Academies Press; 2007.

4 Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: Cancer incidence, mortality, and prevalence worldwide: International Agency for Research on Cancer; 2003.

5 Boyle P, and Levin, B. World Cancer Report 2008. Lyon: International Agency for Research on Cancer; 2008.

6 Kanavos P. The rising burden of cancer in the developing world. Annals of Oncology 17 (Supplement 8): viii15–viii23, 2006.

7 González Pier, E, Gutiérrez-Delgado C, Stevens G, et al. Priority setting for health interventions in Mexico's System of Social Protection in Health. Lancet 2006; 368: 1608–18.

8 Comisión Nacional de Protección Social en Salud. Sistema de protección social en salud: informe de resultados, enero–junio, 2010. http://www.seguro-popular.gob.mx/images/contenidos/Informes_Resultados Informe_Resultados_1sem2010.pdf

9 Institute of Medicine. The US commitment to global health: recommendations for the public and private sectors. Washington, DC: National Academies Press, 2009.

10 Frenk J. Rethinking the institutional architecture for global health. Institutions for Closing the Knowledge-Action Gap in Global Health; John F Kennedy School of Government, Harvard University, Cambridge, MA, USA; June 11–13, 2008.

11 Sepúlveda J, Bustreo F, Tapia R, et al. Improvement of child survival in Mexico: the diagonal approach. Lancet 2006; 368: 2017–27.

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Rationale For and Results to Date from Proton Beam Radiation Therapy

Herman Suit

Abstract

The rationale for use of proton radiation therapy is that by proton beams a superior dose distribution feasible is readily achieved for nearly all anatomic sites. This is based of the law of physics that the range of a proton in a material is finite. Hence, beams can be designed that have a uniform dose across the target and virtually zero dose deep to the target and a modestly lower dose proximal to the target relative to that of a high energy x-ray beam.

Published results by proton therapy have been obtained that are judged superior to those for x-ray therapy for uveal melanoma, chondrosarcoma and chordoma of the skull base, chordoma of sacrum, squamous cell carcinoma and adenocystic carcinoma of the H/N region and hepatocellular carcinoma.

^{12}C ion therapy provides similar biologically effective dose distributions to those by proton beams. There is, however, a modest dose distribution advantage for ^{12}C ion beams in their more narrow penumbra or dose fall-off at the lateral edge of the beam. Of serious interest is the potential of a clinical gain due to the high LET of ^{12}C ion beams. Results that have been published for ^{12}C ion therapy appear to yield higher tumor control rates for chordoma of the skull base, mucosal melanoma of the H/N, renal cell carcinoma and early stage prostate carcinoma.

My opinion is that there is no valid rationale for Phase III clinical trials of two low LET beams one of which delivers fewer doses to normal tissues. This refers to trials of x-ray vs proton beams for sites for which comparative treatment plans demonstrate superior dose distribution for proton beams. In contrast, there is a clear and strong rationale for Phase III trials of protons *vs* ^{12}C ions with only one variable, LET. The fractionation and all technical aspect of treatment planning and dose delivery should be identical.

Why Proton Beams in Radiation Therapy?

The single basis for protons rather than x-rays in radiation therapy is quite simple, ie a *superior dose distribution*. That is, for most tumor anatomic situations there is a lesser radiation dose to normal tissues for a defined dose and dose distribution to the target volume than is achievable by the most technologically advanced technique with x-ray beams. The yield is an increased tolerance by the patient of radiation dose and hence higher doses may be administered. The result is a higher tumor control probability [TCP]. A therapeutic gain is a higher TCP for a specified NTCP. Were the TCP judged acceptable, eg $^{3}0.9$, an alternate strategy could be to maximize the reduction of NTCP at an accepted TCP. Thus, there would be a definite clinical gain for either approach.

The superior dose distribution of a proton treatment plan is the result of a law of physics that the range of a proton beam in tissues is finite[1]. That range is a function of the beam's initial energy and the density of the matter in the beam path. A pristine proton beam penetrates a defined material a distance determined by the entrance beam energy and the material density. The penetration depth is highly uniform. For example a beam with a range of 200 mm in pure water, the variation in range of the individual protons is only a few mm **[Michael Goitein, personnel communication, 2010].** Thus, a well designed distribution of proton energies yields a uniform dose across the volume of interest, the Spread Out Bragg Peak or SOBP and virtually zero dose deep to the SOBP. This is shown in **Fig. 1a.** The sharp contrast between the depth dose curves of high energy x-ray beams and a clinical proton beam is illustrated in **Fig.1b.** The red areas indicates the volumes that are irradiated but that are not suspected of

involvement by tumor, *ie* negative or not wanted dose. For very superficial tissues, there would be a thin depth that would receive a higher dose by the proton than by x-ray beams, indicated by the green area**.** This is due to dose build-up over the superficial 5-10 mm of tissue by high energy x-ray beams. For tumors close to the surface, a small portion of the total dose is given by x-ray beams of appropriate energy to reduce surface dose. For all but a small fraction of tumors, treatment is by multiple fields so that dose to superficial tissues is not a major consideration.

Fig.1. a. Depth dose curves of a series proton beams of well selected distribution of energies are at the bottom of the figure. The yield is the uniform dose across the depth of interest, the SOBP at the upper part of the figure. **b.** Depth dose curves of a clinical proton beam and a high energy x-ray beam demonstrating the superior dose distribution of the proton beam.

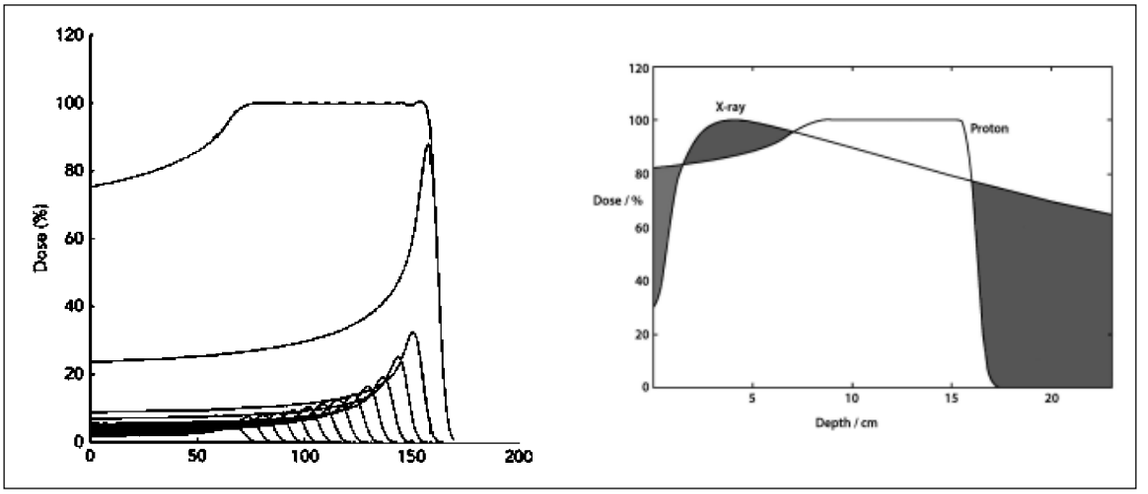

Fig 1.

a Depth dose curves of proton beams of well selected energies to produce the uniform dose across the depth of concern, viz the SOBP. b. Comparison of depth dose curves of a clinical proton beam and a high energy x-ray beam.

A critical point in comparing the two beams is that there is similar flexibility in delivery of proton and x-ray dose. That is, there can be the same number of beams, direction of beams, co-planar or non co-planar, intensity modulated. A recent and elegant technology that can be employed equally by protons and x rays is [4D image guided radiation dose delivery [4D IGRT] **[3]**.

Advances in the technology of radiation oncology have been principally those planned to provide a superior dose distribution. These include the progressively higher energy x-ray beams. These firsts are mentioned: 100 kVp at Jefferson Hospital, Philadelphia in 1907; the 1 MV Van de Graaff machine at the Huntington Cancer Hospital of Harvard University in 1937; the 22 MV Betatron at the University of Illinois in 1948 and the 8 MV linear accelerator in 1953 at Hammersmith hospital, London. **[13]**. These have been accompanied by the introduction of portal imaging, simulators, computer based treatment planning systems, gantries, greatly improved patient positioning, imaging [CT, MRI, PET US and other] and the above mentioned start of 4 D IGRT. These technical advances have generated truly major gains in dose distribution and, hence, clinical outcomes. Our good fortune is that many more are “coming down the pike”. One critical advantage of radiation treatment is that the dose is well localized to the target volume, *ie* quite low doses to tissues not close to the target tissues. This is even more the case for proton irradiation. The total body integrated dose by proton radiation therapy is ~ half that of the high technology x-ray method increasingly employed today, viz intensity modulated x-ray therapy, IMXT **[22]**.

An important additional fact is that a superior dose distribution that reduces radiation dose to chemotherapy sensitive normal tissues permits higher doses of drugs, hence greater anti-tumor effect.

NTCP should be assessed from long term follow-up observations, viz 10-25 years because of the fact that late injures are late. This is also valid for assessing toxicity of chemotherapeutic agents.

A book on the status and potential of proton radiation therapy by DeLaney and Kooy **[7]** has been published, reflecting the widening interest in this technology.

Biological Effectiveness of Clinical Proton Beams Relative to High Energy X-Ray Beams.

This question has been examined is detail using *in vitro and in vivo* systems. J Robertson of the Harvard School of Public Health determined the RBE for 160 MeV protons for the H4 hepatoma cell line *in vitro* at the start of our proton therapy **[28]**. Then came the series of *in vivo* experiments by Tepper etal and Urano etal at the MGH/HCL **[37, 40, 41]** and a goodly number of other investigators employing a spectrum of normal tissues as well as tumors. In 2002, Paganetti etal reviewed the RBE values from all published *in vivo* studies. The result was that the mean RBE [relative to ^{60}Co photons] was 1.1, with no evident dependence of RBE on dose or on tissue investigated **[24].** This value was approximately equivalent to that of 250 kVp x-rays. Accordingly, a proton dose of 70 Gy would be biologically the equivalent of 70 x 1.1 or 77 Gy by a high energy x-ray beam. This RBE value for clinical proton beams has been has been adopted by the International Commission on Radiological Units **[26].** The result has been a much simplified treatment planning. That is, no concern as to the dependence of RBE on dose per fraction, tissue or other factors as is the situation for ^{12}C ion therapy.

Examples of Proton and X-Ray Treatment Plans for Several Anatomic Sites.

Dose distributions are presented in Figs. 2-5 for 4 anatomic sites. The tumor dose has been planned to be the same for the x-ray and proton treatments. These are clear in showing lower dose to normal tissues for comparable tumor doses.

Fig.2 demonstrates the dose distribution for intensity modulated x-ray and proton therapy to a chondrosarcoma of the superior pubic ramus. The intensity modulated treatment method is a comparatively new and very high technology treatment method. **Fig. 3.** shows the dose distribution for treatment of a patient with a skull base tumor, by intensity modulated protons and x rays. Figs. 4 and 5 present treatment plans for elective irradiation pelvic lymph nodes and for a retrobulbar sarcoma. I know that you have seen other examples of proton dose distribution in treatment plans for the pediatric patients in presentations of the excellent work of Tarbell, Yock and associates at earlier SIOP meetings. Mention is made here of a few of their publications on this subject. **[36, 44, 45]**

Figs 2 and 3. Dose distribution for proton and x-ray treatment, using intensity modulated delivery techniques, for a chondrosarcoma of the superior public ramus and a skull base tumor.

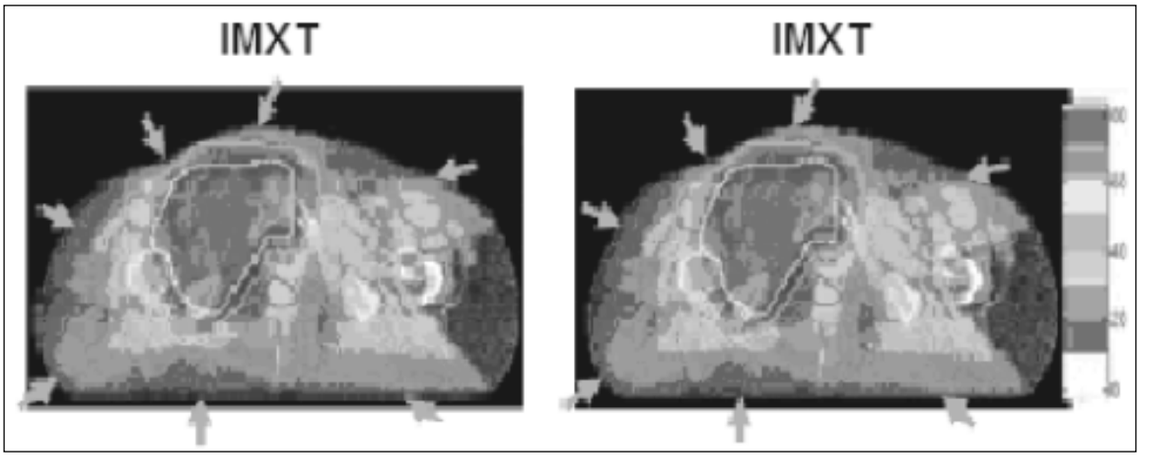

Fig 2a. **Fig 2b.**

a. and b. Dose distribution of an intensity modulated x ray and a proton treatment plan for a chondrosarcoma of the public ramus.

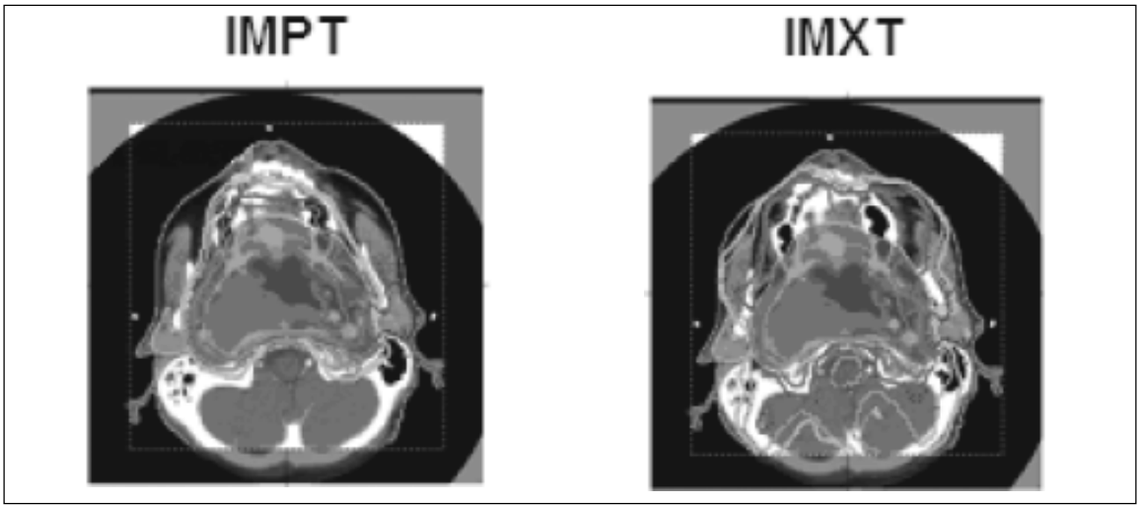

Fig 3a. **Fig 3b.**

a. and b. Dose distribution of an intensity modulated x ray and a proton treatment plan for a tumor of the skull base.

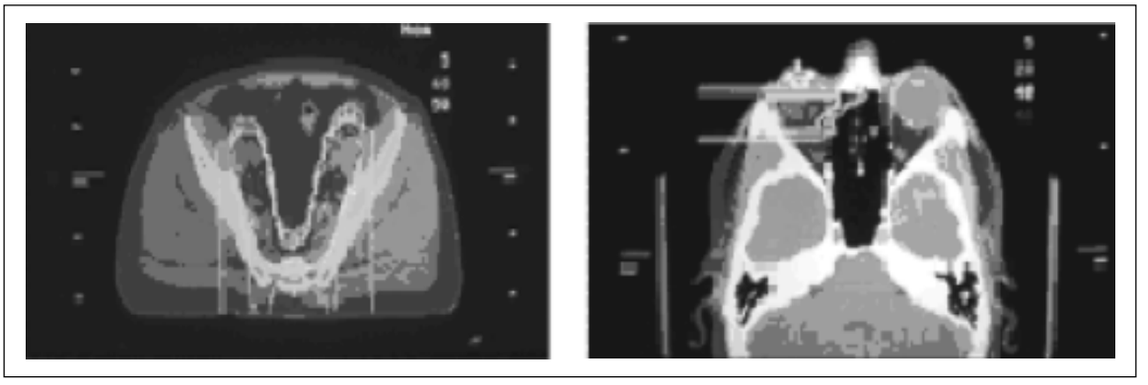

Fig 4a. **Fig 4b.**

a. and b. Dose distribution of a proton treatment plan for a elective irradiation of the pelvic lymph nodes. b. A proton treatment plan of a retrobulbar tumor in a pediatric patient

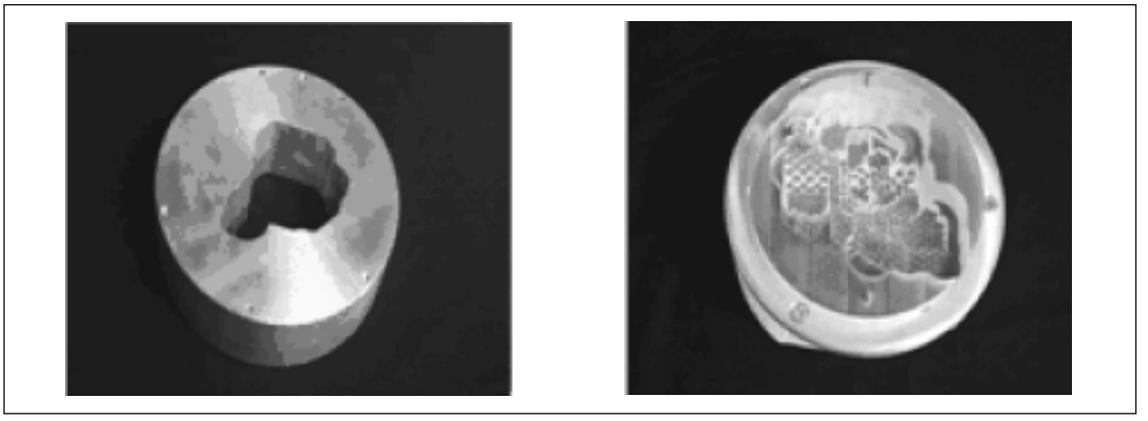

Fig 5a. **Fig 5b.**

a. Beam contour device for one field of proton treatment of a patient. b. The plastic devise made for each field of each patient to provide the planned penetration of the proton beam in each voxel of tissue in the patient.

Figs. 4 and 5. Proton treatment plans for elective irradiation of pelvic nodes and for a retrobulbar tumor.

Clinical Results of Radiation Therapy

The first cancer patient treated by fractionated dose proton beams with intent to cure

My clinical experience before recruitment to the MGH in 1970 had been 2 years a the NCI using the 2 MV Van de Graaff unit. Then for 10 years at the MDACC, I had regular access to the higher energy betatron beams. In my judgment and that of my colleagues was that the higher energy beams were clinically far superior to the 250 x-ray beams. Shortly after coming to the MGH, I was greatly fascinated by the real prospect of clinical study of proton beams for treatment of cancer patients using the Harvard Cyclotron Laboratory [HCL]. Since 1961, there had been an ongoing program of single dose proton irradiation of intra-cranial lesions by the neurosurgical group of R Kjellberg and W Sweet using stereotactic radiosurgery [SRS].

Our proposal in 1971 to use the cyclotron 4 full days per week for proton radiation treatment of cancer patients with the intent to cure was accepted with open enthusiasm by the HCL team. I did not know at the time that the Harvard Physics Department had been planning to close the facility due to the low utilization and high cost. Were our program to be funded, it would be a significant factor in a continued active HCL. Our excellent fortune was to receive NCI grants for the support of this program in 1975. This has continued and recently has developed into a joint program with the proton center at M D Anderson Cancer Center.

Planning commenced in 1971 to prepare for our program. There was impressive talent at the HCL in the persons of A Koehler, Bill Preston and Richard Wilson. In 1972 Koehler and Preston published a paper on the comparative dose distribution of protons, x-ray and electrons **[20].** I had the extremely great fortune of recruiting an exceptional young nuclear physicist from UC Berkeley very early in 1972. He had decided to use his talents in medicine. This was Michael Goitein, a graduate of Oxford University and his Ph D from Harvard[2]. There was initiated a complex series of projects in dose measurements, construction of the patient support system, method for treatment planning and modifying the room in order to treat all body sites.

The proposed strategy was to generate long term survival outcome data relative to that obtained by x-ray treatment. The plan was to have a single variable, namely dose distribution. That is, the dose fractionation would be the same as employed in conventional high energy x-ray therapy, *viz* $\sim$ 2 Gy(RBE)[3].

Treatment of our first patient commenced in Dec 1973 on a 4 year old boy with a large posterior pelvic rhabdomyosarcoma and no detectable metastatic disease. To our knowledge, he was the world's first patient treated by proton beams at standard dose levels per fraction and with the intent to cure. This patient, in treatment position, is shown in **Fig. 6a.** Bi-planar radiographs were taken to determine to the position of the target *viz a viz* the beam. This procedure was repeated until the target was aligned on the beam with high accuracy. Treatment was a combination of HCL protons and betatron x rays of the Boston Medical Center. I had worked with high energy x-rays of a betatron at the MDACC and wanted the same for MGH patients. This was achieved by leasing the betatron for each afternoon. This arrangement continued until our new center was opened in 1975 with an array of linear accelerators. Additionally, the patient had chemotherapy; the effectiveness in 1974 was modest for this category of tumors. There was complete regression and no GI symptoms as there has been only a very low dose to the GI tissues. Regrettably he developed fatal multiple metastatic lesions**.** Of perhaps interest to this meeting is the fact that the last patient treatment at the HCL was also a child. We closed the HCL and transferred to our new proton therapy center at the MGH in 2001, the Francis H Burr Proton therapy Center.

Due to the high TCP for a very large fraction of pediatric carcinomas and sarcomas by the present multi-disciplinary management strategy, the practice is not to raise dose to the evident tumor but to concentrate on techniques to lower doses to normal tissues, *viz* decrease the frequency and severity of late treatment related morbidity. This is the principal interest in proton

therapy for pediatric patients.

Here, selected results of proton treatment of a larger experience with tumors in adult patients are considered.

Skull base chondrosarcoma. Rosenberg et al reported on the MGH series of 200 patients treated by a combination of protons and x rays to a dose of ~72 Gy(RBE)[4] with a 10 year local control rate of 98% **[29]**.

Skull base chordoma. Ares et al**.** have reported a local control rate at 5 years of 81% following 74 Gy(RBE) in the Paul Scherer Institute, near Zurich. They employed the newer proton beam technique of actively scanning of small beams **[1]**. This permits the higher target dose. At the MGH, we utilize passive scanned beams and delivered 69 Gy(RBE) with the lower 5 year local control rate of 59% as reported by Terahara et al **[38].** Dose level is the determinant of TCP of tumors of a specified type, grade and volume.

Sacral chordoma. Delaney et al **[8]** reported that 8 of 9 patients treated by proton radiation alone, *ie* no surgery, for sacral chordoma to 74 Gy(RBE) and had an actuarial 5 year local control of 87%. The one local failure was in a patient treated for a post surgical resection recurrent chordoma.

Uveal melanoma. This was a collaborative program between the HCL, the Massachusetts Eye and Ear Infirmary [MEEI] and our team at the MGH. In 1975 we commenced proton treatment of uveal melanoma[5]. The lesions are small, usually less that 1 cm, and the expectation was that very high doses could be delivered safely and provide a high TCP, despite the general opinion at the time that malignant melanomas were extremely resistant. The first patient to receive proton treatment of a uveal melanoma is shown in the treatment position in **Fig.7.** We started at 10 Gy(RBE) x 5 or 50 Gy(RBE) in 5 days. Shortly, as tolerance appeared to be very high, dose was increased to 14 Gy(RBE) x 5 or 70 Gy(RBE). Results have been good, *viz* local control of 95% at 15 years for the series of 2069 patients as reported by Gragoudas et al. **[18, 19]**. Similar results have been obtained from centers in many countries. For example, Eggers et al in references **10 and11**. reported from Switzerland on a series of 2435 patients with local control of 95% at 10 years. The enucleation rate in proton treated patients has been 3-8% **[35]**. The one report for stereotactic photon treatment using the same dose to the tumor and, hence, the same TCP at 2.9 years, but the enucleation rate was 13% **[9]**.

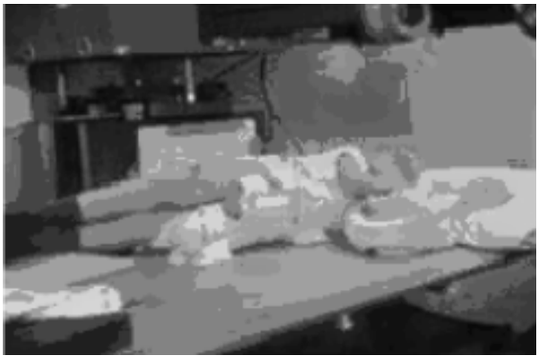

Fig 6a.

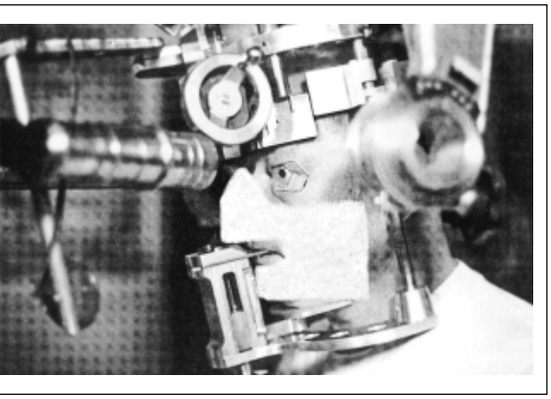

Fig 6b.

a.The first patient treated by low dose per fraction with the intent to cure. This patient was a 4 year old boy with a posterior sarcoma of the pelvis. b. The first patient to be treated by proton beams for a uveal melanoma.

Adenocystic carcinoma of the Head and Neck. In a series of 23 patients with locally advanced adenocystic carcinoma treated to 76 Gy(RBE) at the MGH–HCL by protons, the 3 year local control result was 93%, as described by Pommier et al **[25].**

Squamous cell carcinoma of the Head and Neck. For 29 patients with locally advance squamous cell carcinoma patients were treated to 76 Gy(RBE) at Loma Linda proton center, local control at 5 years was 88% as reported by Slater et al **[32].**

Hepatocellular Carcinoma. From Tsukuba, Japan, the local control at 5 years in their series of 162 patients with hepatocellular carcinoma was reported by Chiba et al. to be 87% **[4].** Mornex et al. in France had local control in 19 of 25 patients at 2.4 years after x-ray treatment and 4 patients developed GIII toxicity **[23]**. This compares with 5 patients among 162 patients at Tsukuba that developed =GII injury after proton therapy and longer follow-up observations

Prostate carcinoma. Results of two phase III proton therapy trials have demonstrated that TCP does increase with dose, no surprise. Shipley et al. **[31]** conducted a Phase III trial of proton therapy on 189 patients with T3-4, N0-2, M0 stage disease and clinical local control as the end-point. Local control rates were 81% and 92% at 5 years at dose levels of 67.2 and 75.6 Gy(RBE), respectively. Treatment was 50.4 Gy pelvic dose by x rays and then a proton boost to 67.2 or 75.6 Gy(RBE). Treatment was radiation alone, *viz* no hormonal therapy.

Results of a Phase III trial have been reported by reported by Zietman et al that was conducted by MGH and Loma Linda for stages T1b-2b prostate cancer. Doses were 70.2 and 79.2 Gy(RBE) to 197 and 196 patients. The 5 year biochemical control rates were 61% and 80%, respectively. **[46].** The treatments were 19.8 or 28.8 Gy(RBE) proton boost dose and then 50.4 Gy by x rays to larger pelvic fields to the pelvis. These patients were treated by radiation alone.

Carbon [^{12}C] Ion beam Therapy

^{12}C ions are also positively charged particles but massive relative to a proton, *viz* by a factor of 12. The clinical interest in ^{12}C ions is based on the fact that their tracks are substantially more densely ionized, *ie* higher liner transfer of energy or LET to the irradiated material. This high LET results in higher biological effectiveness [RBE] than protons. Three radiobiological features of the LET of clinical ^{12}C ion beams may be important for radiation therapy are: 1] a lower oxygen enhancement ratio, *viz* lesser impact of hypoxic regions in tumor tissue on radiation response; 2] a smaller variation in radiation sensitivity with position in the cell replication cycle; and 3] a reduced ability to repair damage from high LET radiation. As of this date, a clinical benefit of high LET radiation has not been demonstrated. Regarding item 3, the available data provide no evidence that tumor cells have an inherently higher sensitivity to high LET radiations than do normal cells. For item 2, this appears to be a minor consideration at the LET values of clinical ^{12}C ion beams. There several tumors that have demonstrated hypoxic regions, *viz* squamous cell carcinoma of H/N region, uterine cervix, prostate adenocarcinoma among others. Clinical studies of H/N squamous cell carcinoma by fast neutron [high LET] and ^{12}C ion beam therapy have not demonstrated higher TCP values than by x-ray or photon beams. Suit et al. have recently reviewed the local control results of proton and carbon therapy **[35].** Meaningful assessment of the efficacy proton and carbon ion therapy is not feasible at present as the proton treatments have been largely based on conventional dose per fraction schedules while carbon ion therapy has been hypofractionated, *viz* large doses per fraction. The actual local control rates as published indicate higher rates by ^{12}C ion therapy for chordoma of the skull base, prostate carcinoma, mucosal melanoma of the H/N region and primary renal cell carcinoma. In contrast, proton therapy results appear to be higher than those by ^{12}C ion therapy for chondrosarcoma of the skull base, squamous cell carcinoma and adenocystic carcinoma of the H/N region. The results for chordoma of the sacrum, uveal melanoma and hepatocellular carcinoma are approximately equivalent. For early stage NSCLC, the short term local control results are similar for x rays, protons and ^{12}C ions as the doses employed are very high for each beam category.

There is an unambiguous need for Phase III trials of ^{12}C ions *vs* protons with only a single variable, LET. That is. the dose fractionation needs to be identical for both arms. There must also be standard technology of treatment delivery, definition of the margin for suspected sub-clinical extension of tumor scoring of local control results, *viz* distinction between local *vs* marginal failure and quality of life. At present there is non-trivial variation in these clearly important factors between the several proton and carbon ion therapy centers.

Are clinical trials of proton therapy *vs* x-ray therapy warranted?

My assessment is that the answer is a clear no,

as discussed in some detail earlier **[33]**. This opinion is based on the fact that comparative treatment plan studies can demonstrate the dose to be delivered to each point in the patient with narrow confidence limits. That is, the stated doses to be delivered are the result of physics and not opinion; they can be and are very frequently measured. Provided that the comparative treatment plan demonstrates a lower dose to normal tissues, a trial for that tumor category is inappropriate. That is, we have approximately 100 years experience in the administration of low LET radiation to human tissues. That radiation can injury normal tissues is a fact. There is no known advantage to any patient to have radiation administered to any tissue. This pertains for doses down to levels that can be measured.

There is no rationale known to me for proposing a long term and costly study to examine if there is an advantage to administering a lower dose of a known toxic agent to uninvolved normal tissues of human patients. Our use of clinical trials and clearly limited resources should be directed to study of important questions. One example would be Phase III trials to determine if high LET radiation treatment of epithelial and mesenchymal neoplasms yields superior results, *viz* a higher TCP for a defined late effect NTCP. Another significant question, what should be the dose and fractionation protocol. Several analyses of late tissue injury following proton therapy have been published, two are mentioned here **[6, 30]**. Clearly more work in this area is warranted. Importantly, there is a need to assess even more than has been performed trials to evaluate to dose and timing of radiation and chemotherapy.

A personal experience while at the MDACC that might be of interest is briefly mentioned. I was recruited from the NCI in 1959 and within a few months, Gilbert Fletcher, Chief of the department called me to his office and stated that it would be good for me to learn a bit of thinking by some of our "colleagues". The NCI had called a meeting to discuss the demand by a number of prominent general radiologists. They were insisting that the federal government not allow any additional ^{60}Co units to be installed in the US until there were clinical studies that demonstrated a clinical gain relative to 250 kVp x-ray beams. Fletcher stated that he would not waste his time going to Bethesda and participate in such a senseless discussion. However, as I was the youngest faculty in the department I should attend. He further opined that the experience would be instructive. The critical fact is that for 10 x 10cm fields of a ^{60}Co and a 200 kVp [1.5mm Cu HVL] beams the dose to the skin is 40% for the ^{60}Co radiation vs 99% for the 250 kVp rays. Further the doses at10cm depth are 58**%** *vs* 36**%** of the surface dose respectively. Additionally the dose to bone in the beam path is substantially lower for ^{60}Co than the 250 kVp radiations. These facts made clear the clinically significant advantages for ^{60}Co irradiation. That is no concepts were one to accept physically measured doses. As Fletcher predicted, there was no perceptible slowing of the installation of the 60Co units after that NCI meeting nor was there any action by the NCI.

Historical Notes

Protons. The proton was discovered in 1919 by E Rutherford at Manchester, England. He did this by bombarding nitrogen gas with alpha particles and observing the ejected protons. The remaining atom had become ^{17}O. This constituted the first man produced alchemy **[34]**. Mention is made that Rutherford much earlier was the first to understand the nature of radioactivity, *ie* there is a change from one element to another or natural alchemy. Further, he stimulated two of his young faculty, Cockcroft and Walton **[5]** at the Cavendish Laboratory at Cambridge to develop much more energetic proton accelerators in order to study to constituents of the atomic nuclei. This was accomplished in 1932 for the bombardment of lithium with energetic protons resulting in 2 helium atoms, *viz* the first artificial alchemy. Accordingly, Rutherford could claim the title as the world's first alchemist.

In 1946, protons and heavier charged particles were proposed for radiation therapy by Robert Wilson a young nuclear physicist at Harvard. This was published in Radiology in 1946 and discussed the rationale quite fully and convincingly **[42]**. Wilson had just returned from several years of important work on the Manhattan project at Los Alamos. Although extremely intelligent and career ambitious[6], he

like many physicists at Los Alamos were deeply concerned that the first military use of the bomb was against civilian targets. He decided that he must make an "atonement for involvement in the development of the bomb at Los Alamos" by making a contribution from nuclear physics that would benefit all of humanity **[43]**. He clearly was successful as evidence of the very large and positive impact of that paper.

The first study of the potential of proton therapy was by C Tobias at UC Berkeley in 1952 **[39]**. The first report of treatment results was by Lawrence in 1957 **[21]** Later carbon, neon and other heavy ion beams were studied at Berkeley. This was followed by programs at Uppsala, Sweden in 1957, at MGH in 1961 at Dubna in1967 physics research center north of Moscow, Moscow in 1969 and St Petersburg in 1975 **[34]**. These programs were almost exclusively single dose treatment of intracranial lesions, stimulated by the success of stereotactic radiation surgery of Leksell and team based on x-ray beams. An important factor for most of these centers was the extremely limited beam time for medical studies.

Protons are of interest also in that there are by a large margin, the most numerous particle in the universe. Namely the estimate is that there are $\sim 10^{79}$ protons and a similar number of electrons **[27]**. The number of neutrons are estimated to be 3×10^{87}, or a ratio of ~3:1 protons to neutrons. Also, perhaps of interest is that the half life of protons is estimated to be $>10^{32}$ years, based on quite extensive experimental studies. Namely to this date not a singe proton decay has been observed. The number of photons is larger than that for proton by a factor of $\sim 10^9$ or a total of some 10^{88} photons in the universe. For the human body, hydrogen atoms are on average estimated to be 63% of the total. The next most common atoms are oxygen at 25.6%, carbon at 9.5% and nitrogen at 1.3% **[2].**

Discussion & Conclusions

Proton treatment plans have superior dose distributions for most anatomic sites relative to the optimal x-ray treatment plans and accordingly provide the basis for increasing dose to the target *viz* increasing TCP. Alternatively, the target dose may be kept constant and attention directed exclusively to decreasing risk of normal tissue injury. Improved local control results appear to have been obtained by proton therapy relative to x-ray therapy of skull base chondrosarcoma and chordoma, chordoma of sacrum, H/N squamous cell carcinoma and adenocystic carcinoma and hepatocellular carcinoma. Additional clinical results at long term follow-up are needed for assessing clinical gains for a larger number of sites studied. With the rapidly increasing number on proton centers, a much more meaningful evaluation of clinical results should be available in 5-10 years. For me, Phase III clinical trials are not warranted for those sites that demonstrate a superior dose distribution by proton beams as both x rays and clinical proton beams are low LET.

Carbon ion beam therapy is of potential clinical benefit due to either the high LET and or the more narrow penumbra. The available data indicate a gain in local control rate relative to proton treatment for chordoma of skull base, prostate carcinoma, renal cell carcinoma and H/N mucosal malignant melanoma. The results appear similar for hepatocellular and early stage NSCLC. There is a need for Phase III trials of proton *vs* carbon ion therapy. The design should feature a single variable, LET. Critical trial design needs to require identical dose fractionation, all technical features of the dose delivery and outcome assessment.

[1] *This applies similarly to heavier ion beams, eg helium, lithium and carbon.*

[2] *Goitein was highly creative and productive. Note that he developed the first computer based treatment planning system used clinically, the technique for "Beam's Eye" view, calculation of uncertainty in the treatment plan and calculation of the impact of density heterogeneity on proton dose distribution and others* ***[14-17]***

[3] *Gy(RBE) is the dose in Gy multiplied by the RBE, ie 1.1.*

[5] *E Gragoudas of the MEEI, the HCL and MGH teams.*

[6] *Robert Wilson was appointed to develop the Fermi Lab in 1967. He did this ahead of schedule and below budget* ***[12]****. He led the development of the first accelerator. In 1977, the Fermi team discovered the Bottom Quark. Wilson laid out the general architectural plans of a truly impressive central building. Widely rated as a structure of architectural beauty. One feature was the openness of the office spaces, viz no closed spaces or solid walls. Additionally he was a sculptor of significant reputation The initial accelerator was replaced by the Tevatron in 1983 as the world's was the largest and most powerful particle accelerator until the Large Hadron Collider began operation near Geneva.*

References

1. Ares C, Hug E, Lomax A, etal. Effectiveness and safety of spot scanning proton radiation therapy for chordomas and chondrosarcomas of the skull base: first long-term report. *Int J Rad Oncol Biol Phys* 2009; 75:1111-18.
2. Atoms in the human body.http://en.wikipedia.org/wiki/Composition_of_the_human_body
3. Chen GT, Kung JH, et al. Four-dimensional imaging and treatment planning of moving targets. Front Radiat Ther Oncol. 2007; 40: 59-71.
4. Chiba T, Tokuue, Matsuzali Y, et al. Proton beam therapy for hepatocellular carcinoma:a retrospective study of 162 patients. Clin Cancer Res. 2005;11:3799-805.
5. Cockcroft J, Walton E. First Splitting of Atom. Cern Currier. 2007. Wikipedia. http://cerncourier.com/cws/article/cern/31864
6. Debus J, Hug EB, et al. Brainstem tolerance to conformal radiotherapy of skull base tumors. *Int J Radiat Oncol Biol Phys* 1997; 39(5): 967-975.
7. Delaney T and Kooy. H. Proton and Charged Particle Radiotherapy. Philadelphia PA, USA., Lippencott Williams & Wilkins. 2008.
8. Delaney T, Liebsch N, Pedlow F, Adams J etal. Phase II Study of High-Dose Photon/Proton Radiation therapy in the Management of Spine Sarcomas. *Int J Radiol Biol Phys. 2009*; 74: 732-9.
9. Dieckmann K, Georg D, Bogner J, etal Optimizing Linac-based stereotactic radiotherapy of uveal melanomas: 7 years' clinical experience. *Int J Rad Oncol Biol Phys. 2006;65:S47-52*
10. Egger E, Schalenbourg A, Zografos L, et al. Maximizing local tumor control and survival after proton beam radiotherapy of uveal melanoma. *Int J Rad Oncol Biol Phys* 2001;51:138–47.
11. Egger E, Zografos L, Schalenbourg A, et al. Eye retention after proton beam radiotherapy for auveal melanoma. *Int J Rad Oncol Biol Phys* 2003;55:867–80.
12. Fermi Laboratory. Wikepedia http://en.wikipedia.org/wiki/Fermilab.
13. Gagliardi R, Wilson J. A History of the Radiological Sciences. Reston, VA: Radiology Centennial; 1996.
14. Goitein M. Compensation for inhomogeneities in charged particle radiation therapy using tomography. *Int J Radiat Oncol Biol Phys* 1978; 4: 449-508.
15. Goitein M Calculation of the uncertainty in the dose delivered in radiation therapy. JAMA. 1980; 244 1347-1350.
16. Goitein M, Abrams M, et al. Multi-dimensional treatment planning: II. Beam's eye-view, back projection, and projection through CT sections. *Int J Radiat Oncol Biol Phys*.1983; 9(6):777-787
17. Goitein M and M. T. Planning proton therapy of the eye. Med. Phys.1983;10: 275-283.
18. Gragoudas E, Wenjun LI, Goitein M. etal. Evidence-based estimates of outcome in patients irradiated for intraocular melanoma. *Arch Opthalmol* 2002;120:1665-71.
19. Gragoudas E. Proton beam irradiation of uveal melanomas: The first 30 years. The Weisenfeld Lecture. IVOS 2006;47: 4666-73.
20. Koehler AM, Preston WM. Protons in radiation therapy. Comparative dose distributions for protons, photons, and electrons. *Radiology*. 1972;104:191-5.
21. Lawrence JH. Proton irradiation of the pituitary. Cancer. 1957;10:795-8.
22. Lomax A, Bortfeld T, Goitein G, etal. A treatment planning inter-comparison of proton and intensity modulated photon radiotherapy. *Radiother Oncol*. 1999; 51 257-71.
23. Mornex F, Hirard N, Bexiat, C etal. Feasibility and efficacy of three dimensional-conformal in cirrhotic patients with small size hepatocellular cca non eligible for curative therapies-mature results of the FrenchPhase II RTF trial. *Int J Rad Oncol Biol Phys*. 2006;66:1152-58
24. Paganetti H, Niemierko A, et al. (2002). Relative biological effectiveness (RBE) values for proton beam therapy. Int J Radiat Oncol Biol Phys.2002;53(2): 407-421.
25. Pommier P, Liebsch NJ, et al. (2006). Proton beam radiation therapy for skull base adenoid cystic carcinoma. *Arch Otolaryngol Head Neck Surg* 132(11): 1242-1249.
26. Prescribing, Recording, and Reporting Proton-Beam Therapy. Report 78, *Journal of the ICRU[International Commission on Radiation Units and Measurements* 2007;7:No. 2.
27. Protons in the observable universe. http://en.wikipedia.org/wiki/Observable_universe
28. Robertson JB, Williams JR, et al. (1975). Radiobiological studies of a high energy modulated proton beam utilizing cultured mammalian cells. *Cancer*. 1975; 35(6):1664-1677.
29. Rosenberg A, Nielsen G, Keel S, et al.. Chondrosarcoma of the base of the skull: a clinicopathological study of 200 cases with emphasis on it's distinction from chordoma. *Am J Surg Path*. 1999;23:1370–8.
30. Santoni R, Liebsch N, et al. Temporal lobe [TL] damage following surgery and high-dose photon and proton irradiation in 96 patients affected by chordomas and chondrosarcomas of the base of the skull. *Int J Radiat Oncol Biol Phys*.1998; 41(1).
31. Shipley W, Verhey L, Munzenrider J, et al. Advanced prostate cancer: The results of a randomized comparative trial of high dose irradiation boosting with conformal protons compared with conventional dose irradiation using photons alone*. Int J Rad Oncol Biol Phys*. 1995;31:3–12.
32. Slater J, Yonemoto L, Mantik D, et al. Proton radiation for treatment of cancer of the oropharynx: early experience at Loma Linda University Medical Center using a concomitant boost technique. *Int J Rad Oncol Biol Phys.* 2005;62:494–500.
33. Suit H, Kooy H, Trofimov A, et al. Should positive phase III clinical trial data be required before proton beam therapy is more widely adopted? No. *Radiother Oncol.* 2008;86:48-53.

34. Suit H, Chu W in Delaney T and Kooy. H. Proton and Charged Particle Radiotherapy. Philadelphia PA, USA. Pp 1-7. Lippencott Williams & Wilkins. 2008.

35. Suit H, DeLaney T, Goldberg S et al. proton vs carbon ion beams in the definitive radiation treatment of cancer patients. *Radiother Oncol*. 2010; 95[1]:3-32.

36. Tarbell NJ, Smith AR, et al. The challenge of conformal radiotherapy in the curative treatment of medulloblastoma. *Int J Radiat Oncol Biol Phys*. 2000;46: 265-266.

37. Tepper J, Verhey L, et al. In vivo determinations of RBE in a high energy modulated proton beam using normal tissue reactions and fractionated dose schedules. *Int J Radiat Oncol Biol Phys.*1977; 2: lll5-ll22.

38. Terahara A, Niemierko A, Goitein M, etal. Analysis of the relationship between tumor dose inhomogeneity and local control in patients with analysis of the relationship between tumor dose inhomogeneity and local control in patients with skull base chordoma. *Int J Radiat Oncol Biol Phys.*1999 Sep 1;45(2):351-8.

39. Tobias CA, Anger HO, Lawrence JH. Radiological use of high energy deuterons and alpha particles. *Am J Roentgenol Radium Ther Nucl Med.* 1952;67:1-27.

40. Urano M, Goitein M, Verhey L, et al. Relative biological effectiveness of a high energy modulated proton beam using a spontaneous murine tumor in vivo*. Int J Radiat Oncol Biol Phys*. 1980;6:1187-93.

41. Urano M, Verhey LJ, Goitein M, et al. Relative biological effectiveness of modulated proton beams in various murine tissues. *Int J Radiat Oncol Biol Phys.* 1984;10:509-14.

42. Wilson R. The Radiological Use of Fast Protons. *Radiology*. 1946; 47:487-91.

43. Wilson R. A Brief History of the Harvard University Cyclotrons. Cambridge, MA: Harvard University Department of Physics; 2004.

44. Yock T and Tarbell. N.. Technology insight: Proton beam radiotherapy for treatment in pediatric brain tumors. .*Nat Clin Pract Oncol* 2004; 1(2): 97-103.

45. Yock T, Schneider R, et al. (2005). Proton radiotherapy for orbital rhabdomyosarcoma: clinical outcome and a dosimetric comparison with photons. *Int J Rad Oncol Bio Phys* 63(4): 1161-1168.

46. Zietman A, De Silvio M, Slater J, etal. Comparison of conventional dose vs high dose conformal radiation therapy in clinically localized adenocarcinoma of the prostate. *JAMA* 2005;294:1233-9.

Translational Research and Surgical Strategies of Childhood Solid Tumors

Dietrich von Schweinitz

Surgery is one of the major elements of therapeutic regimens for malignant solid tumors in childhood besides chemotherapy and irradiation and it represents the mainstay of treatment for benign tumors. Time and extension of tumor resection usually has its fixed place in most pediatric oncologic protocols. However, it is not clear whether this represents the optimal approach in every individual patient due to the large biologic varieties of many of these diseases. The surgeon has to keep in mind that he does not operate on an inanimate object but rather on a living neoplasm in a complicated organism. Therefore, tumor biology should influence the surgical strategy in the individual patient. However, until now there has not been undertaken very much surgery-related biological research on childhood tumors. In this review two questions are asked and answers shown with some examples:

- How can results of translational research influence surgical strategies for distinct tumor entities or individual patients?
- How can surgery itself influence the biological behaviour of childhood tumors?

Influence of translational research on surgical strategies

Molecular prognostic markers and surgical strategies. In recent years a large number of molecular genetic alterations have been detected in childhood malignancies (1). For some of these it could be shown that they are valid prognostic markers and can be used to define patient risk groups for differentiated treatment approaches. This may also effect the surgical strategy in some cases. In **neuroblastoma** a large number of prognostic relevant genetic and molecular genetic alterations have been described. The best known and most powerful of these is the amplification of the *MYCN* oncogene in the tumor cells. Until now there have not been undertaken many investigations analysing whether the amplification of *MYCN* should lead to a special surgical approach. In a large retrospective study on the German Cooperative Neuroblastoma Trials NB79 – 90 with 2251 patients we found that radical surgery with resection of more than 90% of the tumor mass does not improve long-term survival of the majority of patients with a neuroblastoma of any stage, but that those with an INSS stage 3 or 4 tumor with *MYCN* amplification have a better outcome after a radical resection (2). Currently, these results are validated with the data of the recent German study NB97. But the relevance of other well-known prognostic markers in neuroblastoma such as alterations of chromosomes 1p and 11q, Trk A and B, and others is still unknown. In **Wilms tumor** loss of heterozygosity for chromosomes 1p and 16q have been shown to be adverse prognostic factors in favorable-histology tumors, especially when they occur combined (3). Should surgery for these tumors be more radical than in others and should it be done exclusively after neoadjuvant chemotherapy? Can nephron preserving surgery be promoted in favorable-histology Wilms tumors without these molecular genetic alterations? Until now there exist no valid data to answer these questions, nor investigations on other recently found aberrations such as overexpression of the *CACNA1E* transcript (4). For **hepatoblastoma** Cairo et al. (5) very recently found a 16-gene signature with a highly significant prognostic relevance and we identified further putative prognostic molecular markers, but the relevance of these findings for surgery is not known.

Molecular markers for differential diagnosis and surgical strategies. During the last years a large number of cytogenetic and molecular genetic alterations have been identified in **malignant soft tissue sarcomas**. These can

help in establishing the differential diagnosis between these tumors especially in cases with very undifferentiated and unclear histology and immunohistochemistry (6). This will then influence the chosen treatment regimen and should also be taken into account for planning of surgery. It can well make a difference for the surgical approach whether a highly malignant tumor with unclear histology is catagorised as rhabdomyosarcoma, extraskeletal Ewing sarcoma or rhabdoid tumor, since it will determine timing of surgery, as well as the attempted radicality in relationship to the accepted risk for complications and mutilation. New markers will be found in these tumors and prospective studies are needed to determine the relevance of these for surgical strategies.

Invasive growth. Some aggressive malignant tumors such as renal tumors, sarcomas and hepatoblastoma display invasive growth into adjacent organs and blood vessels, which effects surgical strategy and can make the utilisation of special surgical techniques necessary. Some neuroblastomas however, which undergo differentiation from small blue round cell histology to ganglioneuroblastoma during chemotherapy also invade blood vessel walls. In these cases differentiated neuroblastoma cells or ganglion cells can be detected between the layers of vessel walls. It is unknown, what the stimulus for this invasion or migration of the tumor cells can be, but this leads to the situation that during surgery the tumor tissue cannot be cleanly dissected from the blood vessels and the risk for their rupture is highly increased. Therefore, in such tumors a radical tumor resection is often not possible without major injury of important vessels (7). In one of our projects we try to find molecular markers with which these specific neuroblastomas could be identified from a biopsy at the time of diagnosis, making a better planning of the final surgery possible.

Anticancer drug resistance. Multiple drug resistance can be found in many malignant childhood tumors, either existing already at the outset of treatment or becoming apparent during the course of disease. Several molecular mechanisms can be responsible for drug resistance, mainly the inhibiton of drug accumulation in the tumor cells, drug detoxification or altered affinity of the intracellular targets, DNA repair mechanisms and the inhibition of apoptosis (8). The knowledge of drug resistance in a tumor will primarily influence the choice of cytotoxic drugs and maybe the application of chemosensitizers, but it should also be taken into account for planning surgery. Here, resistant tumors may need earlier and more aggressive surgery in comparison to tumors which show a steady response to chemotherapy over a long period. For hepatoblastoma we found that the prognosis is reduced in patients in whom the tumor resection is performed after development of multiple drug resistance (9). Therefore, routine analyses of molecular resistant mechanisms in malignant tumors may enable a better tailoring of surgery in the future.

The influence of surgery on tumor biology

Healing from surgical injury and tumor growth. Surgical operations are always associated with injury of the skin and deaper structures. This implicates the process of wound healing, which develops in several phases: hemostasis, inflammation, proliferation and remodeling. These phases are strictly regulated. Of special interest here is the proliferative phase, which is characterised by angiogenesis, collagen deposition, formation of fibrous tissue and epithelialisation. All these steps are made possible by proliferation of specified cells, which again is inaugurated and controlled by **growth factors**. The most important of these are EGF, TGF-alpha, HGF-SF, VEGF, PDGF, FGF-1 and -2, TGF-beta and KGF. There exists ample research activity on the role of these growth factors and their receptors in healing processes and they are also well known to have a role in many benign and malignant tumors. Also it is well acknowledged that there are many biological similarities between healing processes and tumor growth (10). Thus, it seems obvious that surgery and the consecutive induction of the healing process can also effect the behaviour of eventual residual tumor through the involved growth factors. This can well be an activation of tumor cell proliferation and migration, suppression of apoptosis and angiogenesis. Although it is well known that many malignant and benign tumors show an expression or even an overexpression of the receptors for the above

mentioned growth factors, there exist almost no knowledge on this interaction after tumor surgery. From clinical observation of several cases we know that benign **neurofibromas** in children with neurofibromatosis type I (NF I) can react with rapid growth during the first weeks after incomplete resection to at least the former extension. It has been found that neurofibromas express receptors for several of the growth factors involved in healing, especially those for EGF, VEGF and possibly also PDGF (11). However, the role of these receptors and their ligand for post-surgical growth is still unclear. The same accounts for the question whether incomplete surgery of neurofobromas in NF I patients can maybe even enhance the transformation to malignant peripheral nerve sheet tumors. An early medication with an antiproliferative agent such as rapamycin might be a clinical solution in this situation, but there exist no valid studies on this approach.

Furthermore, fractures as well as surgical trauma of the bones lead to a similar healing process during which the activation of osteoblasts and fibroblasts are important for the formation of new bone. This process is also induced under the influence of growth factors, mainly bone morphogenic proteins (BMP), FGFs, PDGF and TGF-beta. Research activities during the last years revealed that these growth factors also have an influence on proliferation and invasion of **osteosarcoma** cells (12, 13). This may be an explanation for the observation of the development of distant metastases after resection of osteosarcomas, especially in former times, when preoperative chemotherapy was not routinely used in this tumor.

Enhancing tumor cell migration by surgery. Minimal invasive surgery is increasingly utilised for taking biopsies and resection of tumors both in the thorax and the abdomen. This technique is very attractive, since surgical trauma is reduced and recovery faster in comparison to open surgery. However, the biological behaviour of neoplasms may be influenced by setting a pneumoperitoneum or pneumothorax with an artificial high pressure. Recently, it was shown that CO2 pneumoperitoneum increases systemic tumor spread in murine **neuroblastoma** by facilitating tumor cell migration (14). Very little is known about such interactions but the first preliminary results indicate that caution is indicated when using new surgical techniques in malignant childhood tumors.

Organ regeneration and tumor growth. Besides inducing wound healing, partial resection of solid organs can also result in complete or partial regeneration of the organ especially in young children. Thus, surgery induces a process of initial cell proliferation and consecutive tissue organisation, which is again controlled mainly by differentiated expression of growth factors and their receptors. The most prominent example for solid organ regeneration is that of the liver after partial hepatectomy, which in young children starts only some days after surgery and is terminated after 6 – 8 weeks. This process has been intensively investigated during the last 20 years and it is now clear that two mitogenic signals are mainly involved in induction and maintaining hepatocyte proliferation: the hepatocyte growth factor – scatter factor (HGF-SF) with the MET receptor and the epidermal growth factor (EGF) with the EGF-receptor (EGFR) together with the other less prominent EGFR ligands TGF-alpha, heparin binding-EGF and amphiregulin (15). Since in a number of young children with extended hepatoblastoma we had to observe rapid growth of lung metastases and sometimes also of local recurrent tumor after liver surgery, we asked whether the induction of liver regeneration can in parallel also induce tumor cell proliferation. Hepatoblastoma cells, which have many common characteristics with fetal or embryonal liver cells, also show a strong expression of MET and EGFR. In a series of investigations we found a highly increased secretion of HGF-SF in children during the first days after major abdominal surgery and especially after hepatic resections. It also became clear that HGF-SF in vitro leads to a dose-dependent increase of viable tumor cells (16). Further research demonstrated that HGF-SF is a strong mediator of tumor cell scattering and migration (17) and inhibits apoptosis through different intracellular signalling pathways, but alone does not directly enhance proliferation of the tumor cells (18). Since also in hepatocytes both HGF-SF and EGF seem to be necessary for sustained proliferation during liver regeneration, our further research will

concentrate on the combined effect of these and other growth factors on growth of hepatoblastoma and possible approaches to inhibit such interactions. Besides the fact that the phenomenon of surgery stimulated growth of a malignant tumor by induction of regeneration of the tumor's original organ is biologically interesting, this observation is important for planning surgery. We therefore advise that liver resection for extended hepatoblastoma should only be performed after administration of effective chemotherapy. Furthermore, our investigations will show whether other agents targeting important molecular pathways are effective in stopping hepatoblastoma growth and can still be applied peri-operatively to the patients. These can be drugs, which we found to inhibit hepatoblastoma cell proliferation in vitro by blocking the IGF-Akt-mTOR- such as rapamycin (19), the hedgehog- (20), or the WNT-pathway (i.e. non-steroidal anti-inflammatory drugs, NSAID; 21).

Conclusion

Some results of molecular research in tumors of childhood have an impact for the development of better surgical strategies and techniques and therefore translational research should also focus on the surgical relevance of biological findings. Also, surgery itself can influence the biological behavior of some childhood neoplasms and these interactions should become a focus of research activities in pediatric surgical oncology.

References

1. Scotting PJ, Walker DA, Perilongo G. Childhood solid tumours: a developmental disorder. Nat Rev Cancer 5: 481-8, 2005
2. von Schweinitz D, Hero B, Berthold F. The impact of surgical radicality on outcome in childhood neuroblastoma. Eur J Pediatr Surg 12: 402-9, 2002
3. Grundy PE, Breslow NE, Li S, et al. Loss of heterozygosity for chromosomes 1p and 16q is an adverse prognostic factor in favorable Wilms tumor: a report from the National Wilms Tumor Study Group. J Clin Oncol 23: 7312-21, 2005
4. Natrajan R, Little SE, Reis-Filho JS, et al. Amplification and overexpression of CACNA1E correlates with relapse in favorable histology Wilms' tumor. Clin Cancer Res 12: 7284-93, 2006
5. Cairo S, Armengol C, De Reynies A, et al. Hepatic stem-like phenotype and interplay of WNT/beta-catenin and Myc signalling in aggressive childhood liver cancer. Cancer Cell 14: 471-84, 2008
6. Osuna D, de Alava E. Molecular pathology of sarcomas. Rev Recent Clin Trials 4: 12-26, 2009
7. Warmann SW, Seitz G, Schaefer JF et al. Vascular encasemenmt as element of risk stratification in abdominal neuroblastoma. Surg Oncol 2010, Epub ahead of print
8. Kuttesch JF. Multidrug resistance in pediatric oncology. Invest New Drugs 14: 55-67, 1996
9. von Schweinitz D, Hecker H, Harms D, et al. Complete resection before development of drug resistance is essential for survival from advanced hepatobastoma – a report from the German Cooperative Pediatric Liver Tumor Study HB89. J Pediatr Surg 30: 845-52, 1995
10. Dvorak HF. Tumors: wounds that do not heal: similarities between tumor stroma generation and wound healing. N Engl J Med 315: 1650-9, 1986
11. Carroll SL, Stonecypher MS. Tumor suppressor mutations and growth factor signalling in the pathogenesis of NF1-associated peripheral nerve sheath tumors: II. The role of dysregulated growth factor signaling. J Neuropathol Exp Neurol 64: 1-9, 2005
12. Luo X, Chen J, Song WX, et al. Osteogenic BMPs promote tumor growth of human osteosarcoma that harbor differentiation defects. Lab Invest 88: 1264-77, 2008
13. Messerschmitt PJ, Rettew AN, Brookover RE, et al. Specific tyrosine kinase inhibitors regulate human osteosarcoma cells in vitro. Clin Orthop Relat Res 466: 2168-75, 2008
14. Yu Y, Kuebler J, Groos S, et al. Carbon dioxide modifies the morphology and function of mesothelial cells and facilitates transepithelial neuroblastoma cell migration. Pediatr Surg Int 26: 29-36, 2010
15. Michalopoulos GK. Liver regeneration after partial hepatectomy. Critical analysis of mechanistic dilemmas. Am J Pathol 176: 2-13, 2010
16. von Schweinitz D, Faundez A, Teichmann B, et al. Hepatocyte growth factor-scatter factor can stimulate post-operative tumor-cell proliferation in childhood hepatoblastoma. Int J Cancer 85: 151-9, 2000
17. Grotegut S, von Schweinitz D, Christofori G, et al. Hepatocyte growth factor induces cell scattering through MAPK/Egr-1-mediated upregulation of Snail. EMBO J 25: 3524-45, 2006
18. Grotegut S, Kappler R, Tarimoradi S, et al. Hepatocyte growth factor protects hepatoblastoma cells from chemotherapy-induced apoptosis by AKT activation. Int J Oncol 36: 1261-7, 2010
19. Hartmann W, Küchler J, Koch A, et al. Activation of phosphatidylinositol-3'-kinase/AKT signalling is essential in hepatoblastoma survival. Clin Cancer Res 15: 4538-45, 2009
20. Eichenmüller M, Gruner I, Hagl B, et al. Blocking the hedgehog pathway inhibits hepatoblastoma growth. Hepatology 49: 482-90, 2009
21. Koch A, Waha A, Hartmann W, et al. Elevated expression on Wnt antagonists is a common event in hepatoblastomas. Clin Cancer Res 11: 4295-304, 2005

New Interventional Technologies in Radiology

Derek J Roebuck

Introduction

Enormous advances have been achieved in paediatric oncology over the past few decades, largely as a result of productive interdisciplinary and interprofessional collaboration. The radiologist has several roles (Table 1) in the paediatric oncology multidisciplinary team (MDT). It has long been assumed, at least by radiologists, that part of the increase in survival of children with cancer has been due to gradual improvements in diagnostic imaging. The magnitude of this contribution is open to debate.

More recently, interventional radiology (IR), a growing subspecialty within radiology, has had an important role in the management of children with cancer. This development can be expected to increase in the future, following in general terms the path of adult oncology practice, where interventional oncology is sometimes called “the fourth pillar of cancer treatment”.

A few authors have reviewed the topic of paediatric interventional oncology [1-3]. The main IR techniques they have described in children with cancer are image-guided biopsy and regional therapy. There is also, however, an important role for IR in supportive care (including central venous access and enteric feeding) and the treatment of complications of cancer therapy [3].

Table 1. Potential roles of the radiologist as part of the paediatric oncology multidisciplinary team.

Screening
e.g. in children at increased risk of developing Wilms’ tumour
Diagnosis of primary tumour
imaging diagnosis
biopsy planning
image-guided biopsy
preoperative localization techniques for thoracoscopic or open biopsy
intraoperative ultrasound
Staging
Central venous access
Hickman/Broviac catheter insertion
central venous port device insertion
peripherally-inserted central venous catheters (PICCs)
Enteric access
gastrostomy
transgastric jejunal tube insertion
Assessment of response to therapy
tumour response
pre-surgical imaging
post-surgical imaging to detect residual disease
Regional therapy
transarterial techniques
intra-arterial chemotherapy
embolization
chemoembolization
radioembolization
percutaneous ablation techniques
radiofrequency ablation
other techniques (see text)
Surveillance for recurrence
Diagnosis and treatment of complications
aspiration and drainage of fluid collections biopsy
biliary drainage or stenting
nephrostomy or ureteric stenting
Palliative care
pleurodesis for malignant effusions
implantation of epidural port-catheter systems

Image-guided needle biopsy

Although laparoscopic surgery has decreased the invasiveness of the surgical biopsy, percutaneous image-guided needle biopsy is even less invasive and is very well tolerated by children. There are several reasons why it is not more widely used in paediatric oncology, even in hospitals where trained operators and appropriate equipment are available. Firstly, there are theoretical concerns about sampling error, although this may also occur with surgical biopsies, and does not in practice appear to be a major problem for either technique. A few authors have suggested that needle biopsy may be less safe than surgical biopsy, although there is no evidence to support this, and it could well be that needle biopsy is safer [4]. Some paediatric pathologists do not like to interpret needle biopsies, although with increasing experience and new laboratory techniques this is gradually becoming less of a problem. Finally, there is sometimes a reluctance to embrace new techniques in paediatric oncology. It would be too harsh to describe this as neophobia, but perhaps fair to say that paediatric oncologists in general favour techniques with which they have had good experience over new and unfamiliar methods. This is sometimes reflected in treatment protocols, which may mandate a surgical biopsy.

In our institution, almost all suspected tumours arising outside the central nervous system (CNS) are biopsied using an image-guided percutaneous method [5]. Where possible, all patients are discussed at a full MDT before biopsy, to identify patients where a different form of biopsy (or no biopsy at all) may be appropriate. For example, when an abdominal tumour is suspected to arise from an ovary, laparoscopy will usually be a better approach [6].

Accuracy

The accuracy of needle biopsy is usually assessed by review of procedures from one or more institutions, where all types of tumour are considered together. This type of analysis usually produces an accuracy of greater than 90% [5, 7]. It makes more sense, however, to examine different clinical indications separately, as the accuracy of the technique, as well as the balance of risks and benefits, will be different [3]. When this is done, it can be seen that not all biopsies are the same (Table 2).

Table 2. Image-guided diagnosis for different types of paediatric tumour.

The term "accuracy" here refers to the proportion of biopsies in which an accurate pathological diagnosis is possible. The percentage given is a range based on series published in the form of manuscripts or abstracts.

Category	Tumour type	Technique(s)	Accuracy	References
central nervous system tumours		image-guided techniques are rarely applicable		
haematological and related disorders	leukaemia and lymphoma	needle biopsy	76%-80%	[8, 9]
		aspiration of fluid collections	89%	[8]
	Langerhans' cell histiocytosis	biopsy of lymph nodes or other organs	not reported	[10-12]
solid tumours	neuroblastic tumours	biopsy of primary tumour and/or metastases	93%-100%	[5, 13, 14]
	renal tumours	needle biopsy	88%-98%	[5, 15, 16]
	liver tumours	needle biopsy	86%-90%	[5, 17]
	bone tumours	needle biopsy	86%-100%	[5, 11]
	soft tissue tumours	needle biopsy	92-100%	[18, 19]
	thyroid nodules	fine needle aspiration cytology	93%	[20, 21]

Needle biopsy technique

Although various forms of image guidance can be used, in practice almost all tumour biopsies are performed by experienced paediatric radiologists are done with ultrasound. Ultrasound appears to be safer, easier and cheaper than computed tomography, and is certainly much quicker. It also allows the procedure to be performed in an angiography suite, so that a central venous access device can be inserted at the same time (see below).

In general, a coaxial core needle biopsy technique is used. This allows the operator to obtain numerous cores of tissue from different parts of the lesion with a single puncture of the tumour capsule. The biopsy tract can then be occluded by injecting plugs of gelatin foam through the outer needle, in an attempt to reduce the risks of haemorrhage and needle tract seeding [1]. This technique produces excellent quality biopsies for analysis, including a complete range of immunohistochemical and genetic tests, and storage for research if appropriate. Fine needle aspiration cytology (FNAC) is a useful alternative for thyroid nodules [20, 21]. In our hospital, we do not use FNAC for the primary diagnosis of malignancy, but other centres have reported successful results [22-24].

Localization and intraoperative ultrasound

Localization of small lung lesions may be appropriate when they are deep in the lung and consequently impalpable, or if thoracoscopic resection is to be used. A percutaneous image-guided approach can be used to insert a hook wire and/or inject dye into the lesion before resection [1, 25, 26]. Another localization procedure, intraoperative ultrasound, can also be used to identify small lesions (for example in the abdomen or lung) when these cannot be palpated by the surgeon [3].

Regional therapy

Regional therapy, with either transarterial or percutaneous ablative techniques, has become the most important element of interventional oncology in adult practice. Indications for regional cancer treatments in children are still relatively few.

Bland embolization

Transarterial embolization without the use of chemotherapeutic agents has usually been used in benign tumours, to treat symptoms and complications. Examples include infantile haemangioma (to control bleeding) and kaposiform haemangioendothelioma (to treat vincristine-resistant thrombocytopenia). The use of bland embolization has also been reported in some malignant lesions, for example in choroid plexus tumours to reduce blood loss at resection [27] and in life-threatening hepatomegaly caused by neuroblastoma metastases [28].

Intra-arterial (IA) chemotherapy

Intra-arterial (IA) delivery of chemotherapeutic agents has certain important theoretical advantages [29]. Drug exposure to the tumour of up to 1000 times the systemic level may be achieved [29]. This may be important if there is an appropriate relationship between dose and response in the tumour and dose-limiting systemic toxicity. IA chemotherapy is therefore particularly attractive when systemic metastases are unlikely or have been controlled by systemic treatment. It may be used for CNS tumours, with or without disruption of the blood-brain barrier [30, 31]. IA melphalan (given into the ophthalmic artery) is a promising treatment for retinoblastoma [32]. Osteosarcoma is another potential application for IA chemotherapy [33,34].

Other intra-arterial techniques

IA therapy of liver tumours has an important advantage over other sites. Most liver tumours derive most of their blood supply from branches of the hepatic artery; normal liver tissue is supplied mainly by branches of the portal vein. Hepatic artery chemoembolization (HACE), a combination of IA chemotherapy and embolization, is widely used to treat liver tumours in adults. In children, HACE has been used for several types of malignant liver tumour, principally hepatocellular carcinoma and hepatoblastoma [35]. Various protocols have been used [36, 37]. The main indications are to make surgery possible in tumours which would otherwise be unresectable without transplantation, as a bridge to transplantation, and for palliation. Preoperative portal vein embolization may be used to increase the future

liver remnant following partial hepatectomy, as an adjuvant to the first indication [35].

Selective internal radiation (radioembolization)

Radioembolization with yttrium-90 microspheres is a promising alternative to HACE. When the tumour is selectively supplied by the hepatic artery, this technique allows very high radiation doses to be delivered without injuring adjacent normal liver [38]. Selective internal radiation requires equipment which is rarely available in paediatric hospitals, and consequently experience in children is limited [35].

Ablative techniques

Various locally destructive techniques are used for the regional treatment of tumours in adults. Of these, radiofrequency ablation (RFA) is currently the most popular, especially for small liver tumours. RFA has been used in the treatment of hepatoblastoma [39,40], fibrolamellar carcinoma [41] and liver metastases [1,42] in children.

RFA has also been used to treat malignant tumours in lung, soft tissue and bone [41]. Another potential role is the treatment of small renal tumours, for example Wilms' tumours arising in a solitary kidney [43, 44]. The best-established use of RFA in childhood at present, however, is the treatment of benign bone tumours, particularly osteoid osteoma [45], although chondroblastomas have also been successfully treated [46].

Other percutaneous ablative techniques used in adults include cryoablation, percutaneous ethanol injection (PEI) and laser or microwave ablation. These have not been widely used in paediatric oncology, although PEI may have a role in the treatment of hyperfunctioning thyroid nodules in teenagers [47].

Another image-guided regional treatment, high-intensity focused ultrasound (HIFU), has been used for local disease control in osteosarcoma [48]. It is possible that HIFU could be used for other paediatric malignancies.

Supportive care

Central venous access

The modern treatment of cancer in children depends heavily on various forms of central venous (CV) access, including Hickman catheters and central venous port devices. A CV access device should, wherever possible, be placed at the same procedure as the biopsy in children with a high probability of having cancer [9]. There is an increasing tendency for image-guided percutaneous techniques to be used for insertion of CV access devices.

Gastrostomy

Many paediatric oncology patients will require enteric feeding, even if their nutritional status at the start of treatment is normal. Feeding tubes can be inserted using various techniques, including fluoroscopic (non-endoscopic) gastrostomy [49] or transgastric jejunal feeding.

Treatment of complications

IR techniques are often extremely useful in the management of the complications of tumours and their treatment. These include procedures that are more commonly performed in non-oncology patients, such as the aspiration or drainage of fluid collections including ascites, pleural and pericardial effusions and abscesses in various locations, airway stenting [50], nephrostomy [51] and biliary drainage [52-54].

Palliative care

IR techniques may occasionally be helpful in a specifically palliative context. Examples of IR procedures which may be helpful in a palliative context include pleurodesis for malignant pleural effusions [55], insertion of epidural or intrathecal catheters connected to implanted ports for pain management [56], and insertion of permanent biliary stents [52].

Conclusions

The benefits of new interventional procedures in paediatric oncology are gradually becoming recognised. Although the general use of some of these techniques is currently limited by the availability of appropriate equipment and trained operators, there is a clear trend towards the wider use of minimally-invasive image-guided biopsy, and it can be expected that regional therapy will become more important in the future.

References

1. Bittles MA, Hoffer FA (2007) Interventional radiology and the care of the pediatric oncology patient. Surg Oncol 16:229-233.
2. Hoffer FA (2005) Interventional radiology in pediatric oncology. Eur J Radiol 53:3-13.
3. Roebuck DJ (2010) Paediatric interventional oncology. Cancer Imaging:in press.
4. Pinarli FG, Danaci M, Tander B, et al. (2004) Bilateral adrenal cystic neuroblastoma with superior vena cava syndrome and massive intracystic haemorrhage. Pediatr Radiol 34:746-749.
5. Garrett KM, Fuller CE, Santana VM, et al. (2005) Percutaneous biopsy of pediatric solid tumors. Cancer 104:644-652.
6. Roebuck DJ (2010) Genitourinary intervention in children. Pediatr Radiol:in press.
7. Sebire NJ, Roebuck DJ (2006) Pathological diagnosis of paediatric tumours from image-guided needle core biopsies: a systematic review. Pediatr Radiol 36:426-431.
8. Garrett KM, Hoffer FA, Behm FG, et al. (2002) Interventional radiology techniques for the diagnosis of lymphoma or leukemia. Pediatr Radiol 32:653-662.
9. Ehrlich PF, Friedman DL, Schwartz CL (2007) Monitoring diagnostic accuracy and complications. A report from the Children's Oncology Group Hodgkin lymphoma study. J Pediatr Surg 42:788-791.
10. Kumar PV, Mousavi A, Karimi M, et al. (2002) Fine needle aspiration of Langerhans cell histiocytosis of the lymph nodes. A report of six cases. Acta Cytol 46:753-756.
11. Yasko AW, Fanning CV, Ayala AG, et al. (1998) Percutaneous techniques for the diagnosis and treatment of localized Langerhans-cell histiocytosis (eosinophilic granuloma of bone). J Bone Joint Surg Am 80:219-228.
12. Ando A, Hatori M, Hosaka M, et al. (2008) Eosinophilic granuloma arising from the pelvis in children: A report of three cases. Ups J Med Sci 113:209-216.
13. Hoffer FA, Chung T, Diller L, et al. (1996) Percutaneous biopsy for prognostic testing of neuroblastoma. Radiology 200:213-216.
14. Roebuck D, Sebire N, Anderson J, et al. (2005) Image-guided core needle biopsy in children with neuroblastoma [abstract]. Pediatr Blood Cancer 45:398.
15. Roebuck D, Michalski AJ (2003) Core biopsy of renal tumors in children [abstract]. Med Pediatr Oncol 41:283.
16. Vujanic GM, Kelsey A, Mitchell C, et al. (2003) The role of biopsy in the diagnosis of renal tumors of childhood: Results of the UKCCSG Wilms tumor study 3. Med Pediatr Oncol 40:18-22.
17. Roebuck D (2004) Ultrasound-guided core needle biopsy of primary liver tumours in young children [abstract]. Pediatr Radiol 34:S127.
18. Chowdhury T, Barnacle A, Haque S, et al. (2009) Ultrasound-guided core needle biopsy for the diagnosis of rhabdomyosarcoma in childhood. Pediatr Blood Cancer 53:356-360.
19. Roebuck D, Barnacle A (2004) Image-guided percutaneous biopsy of soft tissue masses in children [abstract]. Pediatr Radiol 34:S102.
20. Moslavac S, Matesa N, Kusic Z (2010) Thyroid fine needle aspiration cytology in children and adolescents. Coll Antropol 34:197-200.
21. Izquierdo R, Shankar R, Kort K, et al. (2009) Ultrasound-guided fine-needle aspiration in the management of thyroid nodules in children and adolescents. Thyroid 19:703-705.
22. Drut R, Drut RM, Pollono D, et al. (2005) Fine-needle aspiration biopsy in pediatric oncology patients: a review of experience with 829 patients (899 biopsies). J Pediatr Hematol Oncol 27:370-376.
23. Dave B, Shet T, Ramadwar M, et al. (2006) Cytological evaluation of head and neck tumors in children—a pattern analysis. Diagn Cytopathol 34:434-446.
24. Kilpatrick SE, Ward WG, Chauvenet AR, et al. (1998) The role of fine-needle aspiration biopsy in the initial diagnosis of pediatric bone and soft tissue tumors: an institutional experience. Mod Pathol 11:923-928.
25. Roebuck DJ, Hogan MJ, Connolly B, et al. (2010) Interventions in the chest in children. Tech Vasc Interv Radiol:in press.
26. Martin AE, Chen JY, Muratore CS, et al. (2009) Dual localization technique for thoracoscopic resection of lung lesions in children. J Laparoendosc Adv Surg Tech A 19 Suppl 1:S161-164.
27. Pencalet P, Sainte-Rose C, Lellouch-Tubiana A, et al. (1998) Papillomas and carcinomas of the choroid plexus in children. J Neurosurg 88:521-528.
28. Boztug K, Kiely E, Roebuck DJ, et al. (2006) Successful treatment of MYCN amplified, progressive stage 4S neuroblastoma in a neonate with hepatic artery embolization in addition to multimodality treatment. Pediatr Blood Cancer 46:253-257.
29. Ensminger WD, Gyves JW (1983) Regional chemotherapy of neoplastic diseases. Pharmacol Ther 21:277-293.
30. Jahnke K, Kraemer DF, Knight KR, et al. (2008) Intraarterial chemotherapy and osmotic blood-brain barrier disruption for patients with embryonal and germ cell tumors of the central nervous system. Cancer 112:581-588.
31. Neuwelt EA, Gilmer-Knight K, Lacy C, et al. (2006) Toxicity profile of delayed high dose sodium thiosulfate in children treated with carboplatin in conjunction with blood-brain-barrier disruption. Pediatr Blood Cancer 47:174-182.
32. Abramson DH, Dunkel IJ, Brodie SE, et al. (2008) A phase I/II study of direct intraarterial (ophthalmic artery) chemotherapy with melphalan for intraocular retinoblastoma initial results. Ophthalmology 115:1398-1404, 1404 e1391.

33. Chiu TJ, Wang JW, Chen YJ, et al. (2009) Intraarterial Cisplatin and intravenous adriamycin in nonmetastatic osteosarcoma of the extremities: a single institution experience in Taiwan. Chang Gung Med J 32:72-80.

34. Cullen JW, Jamroz BA, Stevens SL, et al. (2005) The value of serial arteriography in osteosarcoma: delivery of chemotherapy, determination of therapy duration, and prediction of necrosis. J Vasc Interv Radiol 16:1107-1119.

35. Roebuck DJ (2010) Alternative approaches for treatment. In: Zimmermann A, Perilongo G (eds) Pediatric liver tumors. Springer, Berlin, p in press.

36. Arcement CM, Towbin RB, Meza MP, et al. (2000) Intrahepatic chemoembolization in unresectable pediatric liver malignancies. Pediatr Radiol 30:779-785.

37. Malogolowkin MH, Stanley P, Steele DA, et al. (2000) Feasibility and toxicity of chemoembolization for children with liver tumors. J Clin Oncol 18:1279-1284.

38. Lau WY, Ho S, Leung TW, et al. (1998) Selective internal radiation therapy for nonresectable hepatocellular carcinoma with intraarterial infusion of 90yttrium microspheres. Int J Radiat Oncol Biol Phys 40:583-592.

39. Ye J, Shu Q, Li M, et al. (2008) Percutaneous radiofrequency ablation for treatment of hepatoblastoma recurrence. Pediatr Radiol 38:1021-1023.

40. Iannitti DA, Dupuy DE, Mayo-Smith WW, et al. (2002) Hepatic radiofrequency ablation. Arch Surg 137:422-426; discussion 427.

41. Hoffer FA, Daw NC, Xiong X, et al. (2009) A phase 1/pilot study of radiofrequency ablation for the treatment of recurrent pediatric solid tumors. Cancer 115:1328-1337.

42. Goncalves de Oliveira-Filho A, Lopes Miranda M, Leonelli Diz F, et al. (2003) Use of radiofrequency for ablation of unresectable hepatic metastasis in desmoplastic small round cell tumor. Med Pediatr Oncol 41:476-477.

43. Brown SD, Vansonnenberg E, Morrison PR, et al. (2005) CT-guided radiofrequency ablation of pediatric Wilms tumor in a solitary kidney. Pediatr Radiol 35:923-928.

44. Morrison PR, Brown SD, vanSonnenberg E (2005) Pediatric return electrodes for radiofrequency ablation in children. AJR Am J Roentgenol 185:84-85.

45. Rosenthal DI, Hornicek FJ, Torriani M, et al. (2003) Osteoid osteoma: percutaneous treatment with radiofrequency energy. Radiology 229:171-175.

46. Christie-Large M, Evans N, Davies AM, et al. (2008) Radiofrequency ablation of chondroblastoma: procedure technique, clinical and MR imaging follow up of four cases. Skeletal Radiol 37:1011-1017.

47. Tarantino L, Francica G, Sordelli I, et al. (2008) Percutaneous ethanol injection of hyperfunctioning thyroid nodules: long-term follow-up in 125 patients. AJR Am J Roentgenol 190:800-808.

48. Li C, Wu P, Zhang L, et al. (2009) Osteosarcoma: limb salvaging treatment by ultrasonographically guided high-intensity focused ultrasound. Cancer Biol Ther 8:1102-1108.

49. Barron MA, Duncan DS, Green GJ, et al. (2000) Efficacy and safety of radiologically placed gastrostomy tubes in paediatric haematology/oncology patients. Med Pediatr Oncol 34:177-182.

50. Huang IA, Hsia SH, Wu CT, et al. (2004) Combined chemotherapy and tracheobronchial stenting for life-threatening airway obstruction in a child with endobronchial non-Hodgkin lymphoma. Pediatr Hematol Oncol 21:725-729.

51. Meir DB, Inoue M, Gur U, et al. (2004) Urinary diversion in children with pelvic tumors. J Pediatr Surg 39:1787-1790.

52. Roebuck DJ, McLaren CA (2010) Gastrointestinal intervention in children. Pediatr Radiol:in press.

53. Akinci D, Gumus B, Ozkan OS, et al. (2007) Percutaneous management of tumoral biliary obstruction in children. Pediatr Radiol 37:975-980.

54. Roebuck DJ, Stanley P (2000) External and internal-external biliary drainage in children with malignant obstructive jaundice. Pediatr Radiol 30:659-664.

55. Hoffer FA, Hancock ML, Hinds PS, et al. (2007) Pleurodesis for effusions in pediatric oncology patients at end of life. Pediatr Radiol 37:269-273.

56. Queinnec MC, Esteve M, Vedrenne J (1999) Positive effect of regional analgesia (RA) in terminal stage paediatric chondrosarcoma: a case report and the review of the literature. Pain 83:383-385.

Psycho-Oncology: An Evolving Collaboration

Andrea Farkas Patenaude and Mary Jo Kupst

Introduction

Our talk will focus on the simultaneous evolution over the past 40 years of the fields of pediatric psycho-oncology and pediatric oncology. What began as a largely clinical enterprise within what was then called "Death and Dying" or "Thanatology", has developed into a shared appreciation of the application of scientific inquiry to questions about the behavioral, psychosocial, neurocognitive and familial outcomes for children whose lives have been affected by cancer. Improvement in the success of treatments for childhood cancer has changed the focus of our work, has enlarged the scope and nature of our inquiry and has increased the importance of attending to psychological outcomes for children who have lifelong sequelae. It has also led us to appreciate the resilience of children and families who live through a pediatric cancer experience and has focused attention on positive outcomes and ways to intervene which enhance them. We will review how psychological services came to be integrated into pediatric oncology care, will consider the research which now often unites pediatric oncologists and pediatric psycho-oncologists in quality of life outcome studies, and will discuss changes in the topics studied, the characterization of the patients, and the methods used. We will also consider fruitful topics for future research.

The Beginnings

Psycho-oncology has been evidence-based from its origins. We will consider the change from the 1970's to the present in the open communication about cancer to children with the disease. We observe that the increasing openness occurred in concert with the significant improvements in cancer treatment for children over that time period, making discussion of the nature of the disease and the prognosis less painful for all concerned. We review some of the research which supported this transition which was conducted by Dr. Eugenia Waechter, a nurse in San Francisco, and Dr. John Spinetta, a psychologist and founder of the SIOP Psychology Committee. These researchers devised assessment strategies to illustrate that sick children had interpreted the dire nature of their disease from the sadness in their parents' faces and the overheard conversations on their hospital floor. The research utilized projective tests in hospital settings and creative models of hospital rooms which the patients were encouraged to play with. In so doing, the children told us what they knew and that they were, in turn, aware that this was something the grown-ups around them were not willing to discuss. These researchers advocated talking to children about their own health and realized the sense of comfort such communication brought to the children. This early research showed what those of us who work in this field now take for granted, the enormous strength of children in facing even the most dire news if they can be assured that those around them will answer their questions honestly and not abandon them.

Once there was a mandate to talk to children about their illness, it made sense that people whose training included learning how to talk to children about sensitive topics should be part of the treatment team. Across the country and across the world at a few major cancer centers, small teams of 1 or 2 psychologists or social workers and some psychiatrists and some lucky students began to be hired to work with children with cancer and their parents. We were part of the team, though exactly what that meant and how we would work together was something we all had to work out. There were no prior models. As the clinical trust grew, so, too, did the need for research to find out what truly was the psychosocial impact of childhood cancer and

what was truly helpful to patients and their family members. We will detail the changes in clinical care which have led to the possibility of having guidelines for psychosocial care and long-term follow-up which the SIOP Psychology group has pioneered and others have adopted. We will review the development of this new field of Psycho-oncology and will discuss the research on outcomes which came from careful observation of the diversity of patient results in terms of neurocognitive changes, social functioning, and late effects.

With increasing attention to subsets of patients, the group of patients who initially had been studied without much differentiation became sub-divided by age at diagnosis, tumor location, treatment protocol and other variables. When uniformity of the studied group increased, sample size decreased and it became more and more critical that collaboration occurred between psychosocial researchers in different centers so that statistical power could be achieved in research results. Groups of pediatric oncology researchers began to include psychosocial researchers among their midst. Groups like the Children's Oncology Group in the United States (formerly the Children's Cancer Group and the Pediatric Oncology Group) , the Dutch Cancer Society groups, French meetings of pediatric oncologists and their psychologists and many others came together regularly, which led to important collaborations to study the impact of our changing treatments on the lives of our patients. More recently, international collaborations have become more commonplace. Another outcome was the long-awaited longitudinal studies, such as the Childhood Cancer Survivor Study, headed by Les Robison, here in the US which follows more than 10, 000 childhood cancer survivors into adulthood and reassesses them at intervals about a range of topics including some which are psychosocial in nature. Also, from those early days, observations about how to minimize the then considerable misery which childhood cancer patients experienced with nausea and vomiting and the pain of bone marrow aspirations led to early attempts at intervention research, led by Spirito, Zeltzer, Kuttner, and, more recently, Walco. While much of the advance in that area has been pharmacological, our early work with hypnosis, distraction, biofeedback, and relaxation brought us into the treatment rooms, helped us to form links with the medical and nursing staff with common goals of decreasing pain and increasing adherence, and convinced us that not only could we study psychosocial outcomes, but we could also aim to improve them. Non-pharmacological approaches to pain and procedural distress remain important components of treatment.

Our understanding of the neurocognitive impact of cancer and our awareness of the importance of acceptance and reintegration of the post-treatment child into the world of school led to growth in the perspective of the psychosocial teams, now present in more hospitals. Neurocognitive testing and late effects studies encouraged further differentiation. Debbie Waber at the Children's Hospital Boston Department of Psychology did ground-breaking work about the gender-based impact of chemotherapy which led to a change in cancer treatment protocols for leukemia. Thanks to the work of the late Ray Mulhern, Bob Butler, Bart Moore, Donna Copeland, Danny Armstrong and others, we now look at the neurocognitive impact on children with leukemia and brain tumors in terms of specific aspects of functioning including attention, memory, processing speed, visual-motor and executive functioning. Our current awareness of neurocognitive effects of cancer treatment relies not only on assessments of the child's cognitive and social skills but also on inside-the-brain MRI data and inside-the gene determinations which we are linking to differential patterns of cognitive and social functioning in pediatric survivors. Kevin Krull, Donald Mabbott and Kathy Vannatta's SIOP and SIOP-PPO talks here this week concern this work. We know that early and repeated assessment is crucial to identify and ultimately to prevent deterioration of functioning and that such testing should be a right of every patient with childhood cancer. We know, too, that it is not just radiation which interferes with development; surgery-only brain tumor patients also suffer effects of treatment. We understand that neurocognitive functioning is also an intermediate outcome, affecting the child and young adult survivor's quality of life, employment, marital status, happiness, and other psychosocial outcomes.

Throughout the evolution of the field of Psycho-oncology, there has been interest in understanding how children with cancer cope with this experience. Initially, we were blown a bit off course by studies which attributed psychopathological qualities to the reactive depression, anxiety, and somatic focus which showed up when measures meant for general population assessments were given to children with cancer. A more enlightened approach began with the development of quality of life measures that went beyond psychopathology by Jim Varni and Ernie Katz, and resilience measures by Joan Haase and Pam Hinds. These measures allowed us instead to differentiate the majority of children whose physical recoveries led to nearly-normal emotional and social functioning from those whose social interactions, self-concept, optimism and self-efficacy were marked by difficulties. Instead of focusing on psychopathology, we focused on coping, drawing from the stress and coping framework of Lazarus et al., still a commonly accepted model. Yet, coping was difficult to measure, partly because of confusion in what was to be measured, a continuing debate. Is coping a style, a set of strategies, categories of behavior, an independent variable, an outcome? Kupst and Schulman, Koocher in the book with the memorable title, The Damocles Syndrome, Katz, Kellerman, and Siegel conducted studies assessing the coping of children with cancer, pointing the way to more rigorous assessment of psychological factors as they affected the life of the child in treatment for cancer and those who had completed cancer treatment. Spirito and Compas developed coping measures for use in pediatric populations. As these studies emerged, so, too, did the need for not just patient-reported outcomes, but for assessment of the child in their environment at home, at school and with peers. Noll and his group have pioneered creative studies of friendship assessed by peers and teachers within a child's classroom, conducted without the child having to be aware that he or she is the focus of study. Increased recognition of the multi-factorial contributions to coping will lead to research which will hopefully help us to understand when intervention is most effective and what form it should take to maximally improve the lives of children with cancer.

Greater awareness of the child's social networks also focused attention on the widening circles of impact of the child's cancer. Parents (especially single parents), siblings, grandparents' needs and burdens were recognized and assessment tools developed. The potential help which could come from peers, teachers, and specialized summer camp programs was acknowledged and continues to be harnessed and studied for its impact. The isolation which had been a primary source of depression for children with cancer, hospitalized for long periods, is significantly reduced now with television classroom hook-ups, cell phones, Skype connections. We see future uses for telemedicine to prevent some of the interruptive travel, reducing the need for those in rural locations to make so many trips to comprehensive cancer centers. Cell phones, Survey Monkey and other internet programs improve researchers' access to teenagers and young adults as well. Technological advances have improved children's access to serious gaming programs which masquerade as fun, while improving adherence to treatment or understanding of recurrence. In addition, online support for patients is provided by a number of websites including Group Loop and Planet Cancer.

Our increasing understanding of genetics offers new challenges and new possibilities for children from families marked by high rates of familial cancers. Close observation and genetic technology allow us to understand the links between Fanconi's anemia in children and breast cancer in mothers, for example. In turn, hopefully, this will advance our underlying knowledge of etiological mechanisms. Psychosocially, we have much to learn about how children and young adults understand hereditary cancer risk and what impact it has on their health behaviors, especially their adherence to screening or risk-reducing options which reduce their very high lifetime cancer risks. The work of Tercyak, Clarke, Richards, Bradbury, Patenaude, and others focuses on these topics. With international cooperation there is potential to study families with very rare, but highly lethal cancer syndromes, like Li-Fraumeni Syndrome, attributable to mutations in the *p53* gene. Again, we can see how, increasingly, specialization and

cooperation between medical and psychosocial colleagues yields fuller, more useful outcome data.

The influence of culture is still not fully understood as it affects cancer treatment and cancer patients. When patients in our laminar air flow bone marrow transplant rooms were the only places in Children's Hospital Boston where parents could not sleep in a room with their child, at the Karolinska Hospital in Sweden every BMT room had 2 beds, the second one for a parent or spouse. "Swedish people wouldn't have it any other way, " Dr. Per Bolme explained. In Paris, at L'Hopital St. Louis's BMT Unit, fresh fruit, denied to Boston patients as unsanitary, was peeled under ultraviolet light and given to patients. The answer there was that French people believe you have to have good food, including fresh fruit, to heal. Even in our high-tech world of BMT, our personal and cultural expectations infiltrate our response to illness and the formulation of our treatment. We do not sufficiently take note of these expectations as we aim to compare treatments, drug for drug. It is another area where we need to differentiate our patients, while finding the similarities. Genetics will help us to understand the physical differences which in some cases guide patient response, but culture must not be forgotten in its influence on healing and hope. As access to complicated cancer protocols becomes more open to a greater diversity of patients, we have to enlarge the umbrella to be able to include non-English speaking (or non-French or non-Dutch depending on where the study is carried out) parents and patients in our psychosocial studies to be sure we understand all operative factors, to see the variety of ways in which resilience flourishes, and anxiety is managed and longterm expectations set.

Our discussion will also look ahead to questions of how we can better translate psychosocial research into useful clinical intervention, predictions of what psycho-oncologists of the future will study and of how we will better confront discrepancies in outcomes across socio-economic and international boundaries. And, finally, we will address the questions of who shall we train in the future and how shall we accomplish the goal of passing on to a new generation of researchers the thrill and challenge which the field of Psycho-oncology offers.

References

1. Alderfer M.A., Mougianis I., Barakat L.P., Beele D., DiTaranto S., Hwang W.T., Reilly A.T., Kazak A, E. (2009). Family psychosocial risk, distress, and service utilization in pediatric cancer: predictive validity of the Psychosocial Assessment Tool. *Cancer* 115, 4339-49.
2. Armstrong, F.D., & Mulhern, R.K., (1999). Acute lymphoblastic leukemia and brain tumors. In R.T. Brown (Ed.) *Cognitive aspects of chronic illness in children* (pp. 47-77). New York: Guilford Press.
3. Bradbury AR, Patrick-Miller L, Pawlowski K, Ibe CN, Cummings SA, Olopade OI, Daugherty CK. (2008). Should genetic testing for BRCA1/2 be permitted for minors? Opinions of BRCA mutation carriers and their adult offspring. *Am J Med Genet C Semin Med Genet.* 148C, 70-7.
4. Brown RT, Wiener L, Kupst MJ, Brennan T, Behrman R, Compas BE, David Elkin T, Fairclough DL, Friebert S, Katz E, Kazak AE, Madan-Swain A, Mansfield N, Mullins LL, Noll R, Patenaude AF, Phipps S, Sahler OJ, Sourkes B, Zeltzer L. (2008). Single parents of children with chronic illness: an understudied phenomenon. *J Pediatr Psychol.* 33, 408-21.
5. Butler, R.W., Hill, J.M., Steinherz, P.G., Meyers, P.A., & Finlay, J.L. (1994). The neuropsychologic effects of cranial irradiation, intrathecal methotrexate, and systemic methotrexate in childhood cancer. *Journal of Clinical Oncology,* 12, 2621-2629.
6. Butler, R.W., Copeland, D.R., Fairclough, D.L., Mulhern, R.K., Katz, E.R., Kazak, A.E., Noll, R.B., Patel, S.K., & Sahler, O.J. (2008). A multi-center randomized clinical trial of a cognitive remediation program for childhood survivors of a pediatric malignancy. *Journal of Consulting and Clinical Psychology*, 76, 367-378.
7. Butler, R.W. & Mulhern, R.K. (2005). Neurocognitive interventions for children and adolescents surviving cancer. *Journal of Pediatric Psychology,* 30, 65-78.
8. Clarke A. (2010). What is at stake in the predictive genetic testing of children? Familail Cancer 9, 19-22.
9. Conner-Smith, J.K., Compas, B.E., Wadsworth, M.E., Thomsen, A.H. & Saltzman, H. (2000). Responses to stress in adolescence: Measurement of coping and involuntary stress responses. *Journal of Consulting and Clinical Psychology*, 68, 976-992.
10. Copeland, D.R., Fletcher, J.M., Pfefferbaum, B., Jaffe, N., Reid, H. and Maor, M (1985). Neuropsychological sequelae of childhood cancer in long-term survivors. *Pediatrics,* 75, 745-753.
11. Copeland, D.R., Moore, B.D., Francis, D.J., Jaffe, N., & Culbert, S. J. (1996). Neuropsychologic effects of chemotherapy on children with cancer. A longitudinal study. *Journal of Clinical Oncology,* 14, 2826-2835.
12. Haase, J.E., Heiney, S.P. Ruccione, K., & Stutzer, C. (1999). Research triangulation to derive meaning based quality-of-life theory: Adolescent resilience model and instrument development. *International Journal of Cancer*, Suppl. 12, 125-131.

13. Hinds, P.S., Gattuso, J.S., Fletcher, A., Baker, E., Coleman, B., Jackson, T. et al. (2004). Quality of life as conveyed by pediatric patients with cancer. *Quality of Life Research*, 13, 761-772.

14. Howlett N.G., Taniguchi T., Olson S., Cox B., Waisfisz Q., De Die-Smulders C., Persky N., Grompe M., Joenje H., Pals G., Ikeda H., Fox E.A., D'Andrea A.D. (2002). Biallelic inactivation of BRCA2 in Fanconi anemia. *Science* 297, 606-609.

15. Katz, E.R., Kellerman, J., & Siegel, S.E. (1980). Behavioral distress in children with cancer undergoing medical procedures. *Journal of Consulting and Clinical Psychology*, 48, 356-365.

16. Koocher, G. P. & O'Malley, J. E. (1981). *The Damocles syndrome: Psychological consequences of surviving childhood cancer.* New York, McGraw-Hill.

17. Kupst, M.J & Bingen, K. (2006). Stress and coping in the pediatric cancer experience. In R.T. Brown (Ed.) *Comprehensive handbook of childhood cancer and sickle cell disease.* (pp. 35-52) . New York: Oxford University Press.

18. Kupst, M.J., Natta, M.B., Richardson, C.C., Schulman, J.L., Lavigne, J.V. & Das, L. (1995). Family coping with pediatric leukemia: 10 years after treatment. *Journal of Pediatric Psychology*, 20, 601-607.

19. Kuttner, L., Bowman, M., & Teasdale, M. (1988). Psychological treatment of distress, pain and anxiety for young children with cancer. *Journal of Developmental and Behavioral Pediatrics*, 9, 374-381.

20. Lazarus, R.S., & Folkman, S. (1984). *Stress, appraisal, and coping.* New York, Springer.

21. Mulhern, R. K., Fairclough, D. & Ochs, J. (1991). A prospective comparison of neuropsychologic performance of children surviving leukemia who received 18-Gy, 24-Gy, or no cranial irradiation. *Journal of Clinical Oncology,* 9, 1348-1356.

22. Mulhern, R. K., Reddick, W.E., Palmer, S.L., Glass, J., Elkin, D., Kun, L.E. et al. (1999). Neurocognitive deficits in medulloblastoma survivors and white matter loss. *Annals of Neurology*, 46, 834-841.

23. Mulhern, R.K. & Butler, R.W. Neuropsychological late effects. In R.T. Brown (Ed.) *Comprehensive handbook of childhood cancer and sickle cell disease.* (pp. 262-278). New York: Oxford University Press.

24. Noll, R.B., Gartstein, M.A., Vannatta, K., Correll, J., Bukowski, W.M., Davies, W.H. (1999). Social, emotional, and behavioral functioning of children with cancer. *Pediatrics,* 103, 71-78.

25. Patenaude A.F. (2003). Pediatric psychology training and genetics: What will twenty-first century pediatric psychologists need to know? *Journal of Pediatric Psychology* 28, 135-145.

26. Patenaude A.F. & Kupst M.J. (2005). *Psychosocial functioning in pediatric cancer. J Pediatr Psychol*. 30, 9-27

27. Reiter-Purtill, J., Vannatta, K., Gerhardt, C.A., Correll, J., Noll, R.B. (2003). A controlled study of the social functioning of children who completed treatment of cancer. *Journal of Pediatric Hematology/Oncology,* 25, 467-473

28. Richards, M.P.M. The new genetics: Issues for social scientists. *Social. of Health and Illness* 15, 567-586.

29. Robison L.L., Armstrong G.T., Boice J.D., Chow E.J., Davies S.M., Donaldson S.S., Green D.M., Hammond S., Meadows A.T., Mertens A.C., Mulvihill J.J., Nathan P.C., Neglia J.P., Packer R.J., Rajaraman P., Sklar C.A., Stovall M., Strong L.C., Yasui Y., Zeltzer L.K. (2009). The Childhood Cancer Survivor Study: a National Cancer Institute-supported resource for outcome and intervention research. *J Clin Oncol.* 27, 2308-18.

30. Spinetta, J. J. (1977). Adjustment in children with cancer. *Journal of Pediatric Psychology,* 2, 49-51.

31. Spinetta, J.J., Rigler, D. & Karon, M. (1974). Personal space as a measure of a dying child's sense of isolation. *Journal of Consulting and Clinical Psychology*, 42, 751-756.

32. Spirito, A., Stark, L.J., & Williams, C. (1988). Development of a brief checklist to assess coping in pediatric patients. *Journal of Pediatric Psychology*, 13, 555-574.

33. Tercyak K.P (2009). Introduction to the special issue: psychological aspects of genomics and child health. *J Pediatr Psychol.* 34, 589-95.

34. Varni, JW., Burwinkle, T.M., Katz, E.R., Meeske, K., & Dickinson, P. (2002). The PedsQL in pediatric cancer: Reliability and validity of the Pediatric Quality of Life Inventory Generic Core Scales, Multidimensional Fatigue Scale, and Cancer Module. *Cancer,* 94, 2090-2106.

35. Waber D.P., Urion D.K., Tarbell N.J., et al. (1990). Late effects of central nervous system treatment of childhood acute lymphoblastic leukemia are sex-dependent. *Dev Med Child Neurol* 34, 64–74.

36. Waechter, E.H. (1971). Children's awareness of fatal illness. *American Journal of Nursing,* 71, 1168-1172.

37. Walco, G.A., Sterling, C.M., Conte, P.M., Labay, L.E., Engel, R., & Zeltzer, L.K. (2005) . Procedural distress in children with cancer: Self-report, behavioral observations, and physiological parameters. *Clinical Journal of Pain,* 21, 484-490.

38. Walco, G.A., Cassidy, R.C. & Schecter, N.L. (1994) , Pain, hurt, and harm. The ethics of pain control in infants and children. *New England Journal of Medicine*, 331, 541-544.

39. Zeltzer, L.K. & LeBaron, S. (1982). Hypnosis and nonhypnotic techniques for reduction of pain and anxiety during painful procedures in children and adolescents with cancer. *Journal of Pediatrics,* 101, 1032-1035.

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INTERNATIONAL SOCIETY
OF PAEDIATRIC ONCOLOGY

New Drug Development for Children with Cancer

Peter C. Adamson

Abstract

Since the introduction of chemotherapy more than 50 years ago, the prognosis of childhood cancer has improved dramatically, with overall 5-year survival rates approaching 80%. Despite these advances, several childhood cancers still have unacceptably low cure rates, and even when treatment is successful, the acute and long-term morbidity of current therapy can be substantial. Over the past decade, progress in our ability to improve the outcome for children with cancer has slowed significantly. We are, however, entering an era with the potential to understand the molecular basis of all childhood cancers in a timeframe previously unimaginable. At a national level, however, our cancer clinical trials infrastructure faces a number of challenges, most notably the ability to move new ideas forward towards successful clinical trials in a timely manner. Our clinical trial resources will need to be primarily focused on diseases with poor to moderate outcome where there is a clear rationale to investigate a relevant targeted new agent. Coupled to this challenge will be to design trials that can clearly isolate the effect of a new agent under study. The most significant near term change may well be in the design of phase II trials, with incorporation of randomized designs needing to be increasingly utilized. Academic centers, government, industry and patient advocates must work together towards a common goal of leveraging discoveries into improved outcomes for all children with cancer.

Since the introduction of chemotherapy for the treatment of childhood leukemia more than 50 years ago, (1) the prognosis of childhood cancer has improved dramatically. The 5-year survival rate for childhood cancers, many of which were uniformly fatal in the pre-chemotherapy era, was 80% for all forms of childhood cancer diagnosed between 1996 and 2004. (2) This improvement in survival is a result of the incorporation of anticancer drugs into treatment regimens that previously relied only on surgery or radiotherapy for the primary tumor. The multimodality approach, which integrates surgery and radiotherapy to control local disease with chemotherapy to eradicate systemic disease, has become the standard approach to treating most childhood cancers.

Despite these advances, several childhood cancers still have unacceptably low cure rates, (3) and even when treatment is successful, the acute and long-term morbidity of current therapy can be substantial. (4,5) As detailed in recent report based on data from the Surveillance, Epidemiology and End Results (SEER) Program, over the past decade progress in our ability to improve the outcome, as measured by the overall mortality rate, has slowed substantially for children with cancer, most notably for children with solid tumors. (6)

Over the past 35+ years, the overarching strategic approach for most childhood cancer treatment, intensification of therapy, is no longer yielding meaningful improvement in survival. As is well known to pediatric oncologists, children who receive standard dose-intensive chemotherapy have greater than an 80% chance of having at least one drug-related toxicity that is severe, life threatening or fatal over the course of their treatment, (7) and the late effects of cancer treatment, including permanent organ and tissue damage, hormonal and reproductive dysfunction and second cancers, are of special concern. Perhaps more startling is a recent report that, relative to the general population, the overall life expectancy for survivors of childhood cancer is shortened by 10 years. (8) Thus the development of new anticancer drugs must be a priority for childhood cancer basic, translational and clinical researchers.

We are entering an era of an unprecedented pace of discovery in cancer research. Costs associated with whole genome sequencing and related methods are falling precipitously, and thus our ability to understand the molecular basis of all childhood cancers is potentially within reach in a timeframe unimaginable just five years ago.

It is likely that a number of molecular targets will be defined for which there are therapeutic approaches currently available or in clinical development for adult cancers. The key question will shift from the laboratory back to the bedside as we ask how we will leverage this knowledge into an improved outcome for children with cancer.

At a national level, our cancer clinical trials infrastructure faces a number of challenges. A recent report from the Institute of Medicine (IOM), "A National Cancer Clinical Trials System for the 21st Century: Reinvigorating the NCI Cooperative Group Program," details the current problems and suggests pathways forward (9). The one pediatric group funded by the National Cancer Institute (NCI), the Children's Oncology Group, does not share all the challenges facing the nine adult cancer cooperative groups. Yet an overarching theme of the report, the ability to move new ideas forward towards successful clinical trials in a timely manner, is indeed a common challenge. Thus if we are to capitalize on the era of discovery, we must fully re-evaluate how we develop novel therapeutic approaches for children with cancer, and in doing so, re-invent the approach to our cancer clinical trials system.

Over the past decade, the most active area of cancer drug development has been in agents that target signaling pathways, most notably tyrosine kinase (TK) pathways. Tyrosine kinases are a family of enzymes that are responsible for transferring phosphate residues from ATP to the hydroxyl group of tyrosine; the phosphorylation of their intended target can lead to a wide array of actions, including cell division, migration, and upregulation of cellular metabolism. (10) The majority of TKs are transmembrane glycoproteins that dimerize upon ligand binding (receptor tyrosine kinase, RTK); others are cytosolic or nuclear non-receptor TKs that are triggered downstream by RTKs. (11) The vital function of TKs makes them ideal targets for

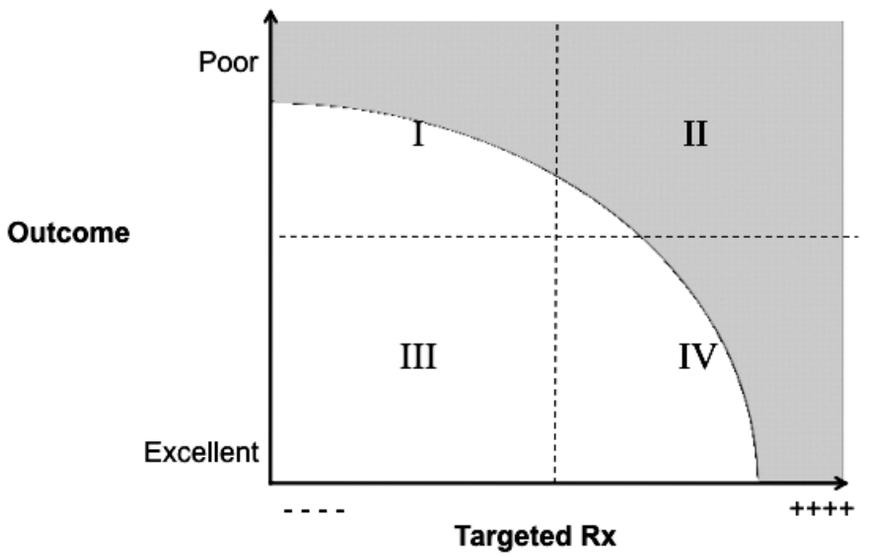

Figure 1.

A re-alignment of clinical trial resources will need to occur if we want to better leverage discovery into improved outcome. Over the past 15 years significant resources have been focused in quadrant III: diseases for which the outcome is relatively good but the ability or availability of targeted new agents is limited to non-existent. We must shift resources primarily to quadrant II: diseases for which the outcome is moderate to poor but our ability to deliver a relevant targeted new agent is reasonably high. As we transition resources, there will still be a need to launch clinical investigations for diseases with very poor outcomes despite a limited knowledge of the molecular basis of disease; moreover, strong consideration should be given to diseases with reasonably good outcomes where a highly relevant targeted therapy is developed (shaded areas).

oncogenic mutations, and altered TK function via proto-oncogene transformation, overexpression, translocation, or deletion contributes to the malignant potential in several human cancers. For example, tyrosine kinase inhibitors currently in clinical use which have or are poised to undergo early phase testing in children (12) include agents that target BCR-ABL — imatinib mesylate, nilotinib and dasatinib; agents that target the epidermal growth factor receptor (EGRF) — erlotinib, geftinib and cetuximab; and agents that target vascular endothelial growth factor (VEGF) — bevacizumab, sunitinib and sorafenib. A wide spectrum of other TKIs are in various stages of clinical development, including agents that target the insulin growth factor receptor (IGFR-1), anaplastic lymphoma kinase (ALK), protein kinase B (AKT), and numerous other pathways. The remarkable homology in the kinase domain of both receptor and cytoplasmic tyrosine kinases has indeed provided a pharmacological opportunity that the biopharmaceutical industry has vigorously pursued. Targets beyond enzyme associated signaling pathways, however, have proven more elusive to therapeutic development.

Although our current understanding of the molecular basis for childhood cancers is variable, we can anticipate a rapid increase in this understanding. A primary limitation on therapeutic advance will be availability of agents capable of effectively impacting key targets. Thus, when coupled to our current knowledge of the value of relevant molecular targets, one key set of factors that must be considered in re-aligning our prioritization of clinical trials is the ability, and availability, of targeted new agents. Of equal importance is the current outcome for the cancer sub-populations being considered for clinical investigation. There are clearly a number of childhood tumors that have not benefitted from any meaningful therapeutic advance for many years, and in the absence of a better understanding of their molecular basis, we will likely need to continue rational, but largely empiric, approaches to clinical trials. A high level view of the approach needed is shown in the Figure. Ideally, our resources should be focused on diseases with moderate to poor outcome where our ability to deliver a relevant targeted new agent is high. Conversely, diseases with good to excellent outcomes, with either a limited understanding or ability to administered targeted agents, should not be a near term focus of clinical investigation resources.

Another key challenge will be to design trials that can clearly isolate the effect of the new agent under study. We can no longer afford to conduct large-scale trials that compare regimens that do not afford a clear understanding of the basis for improvement in outcome beyond the comparison of the regimens themselves. Our clinical trial designs must be able to clearly define the effect of therapy that impacts a specific target. Such designs will potentially allow for an extrapolation of results beyond a fixed regimen.

Perhaps the most significant challenge will be in our design of phase II trials. Demonstration of significant single agent clinical activity in a relapsed population will likely continue to be the most reliable mechanism to advance a new agent to further investigation. For many agents, however, there may be a strong inclination to combine the novel agent with more traditional active but non-curative cytotoxic agents. Interpretation of such trials is inherently difficult and fraught with error. Pursuit of randomized phase 2 trials, including trials that compare distinct targeted agents in conjunction with a common cytotoxic chemotherapeutic regimen, will be but one novel design approach that merits pursuit.

Lastly, we must better position programs to foster enhanced collaboration. The ability to develop and execute clinical trials in a timely manner will greatly enhance our ability to partner with biopharmaceutical partners. Our already small disease populations will become smaller as the molecular basis for these cancers dissect the historic pathologic classifications of disease into sub-populations potentially requiring distinct targeted therapies. Thus developing infrastructures that allow for better international collaborative studies is essential. Moreover, exploration of novel designs that can yield interpretable results with smaller populations will be important.

The next 10 years will be both exciting and challenging. Our approach to clinical trials must evolve in concert with the discoveries made in our laboratories. Academic centers, government,

industry and patient advocates must work together towards a common goal of leveraging discoveries into improved outcomes for all children with cancer.

References

1. Farber S, Diamond LK, Mercer RD, et al. Temporary remissions in acute leukemia in children produced by folic acid antagonist 4-aminopteroylglutamic acid (aminopterin). N Engl J Med. 1948;28:787-93. PMID
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer Statistics, 2009. CA Cancer J Clin. 2009 Jun 25;59:225-49. PMID 19474385
3. Adamson PC, Blaney SM. New approaches to drug development in pediatric oncology. Cancer J. 2005 Jul-Aug;11(4):324-30. PMID 16197722
4. Bhatia S, Meadows AT. Long-term follow-up of childhood cancer survivors: future directions for clinical care and research. Pediatr Blood Cancer. 2006 Feb;46(2):143-8. PMID 16317758
5. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, Friedman DL, Marina N, Hobbie W, Kadan-Lottick NS, Schwartz CL, Leisenring W, Robison LL. Chronic health conditions in adult survivors of childhood cancer. N Engl J Med. 2006 Oct 12;355(15):1572-82. PMID 17035650
6. Smith MA, Seibel NL, Altekruse SF, Ries LA, Melbert DL, O'Leary M, Smith FO, Reaman GH. Outcomes for children and adolescents with cancer: challenges for the twenty-first century. J Clin Oncol. 2010 May 20;28(15):2625-34. PMID 20404250
7. Crist WM, Anderson JR, Meza JL, Fryer C, Raney RB, Ruymann FB, Breneman J, Qualman SJ, Wiener E, Wharam M, Lobe T, Webber B, Maurer HM, Donaldson SS. Intergroup rhabdomyosarcoma study-IV: results for patients with nonmetastatic disease. J Clin Oncol. 2001 Jun 15;19(12):3091-102. PMID 11408506
8. Yeh JM, Nekhlyudov L, Goldie SJ, Mertens AC, Diller L. A model-based estimate of cumulative excess mortality in survivors of childhood cancer. Ann Intern Med. 2010 Apr 6;152(7):409-17, W131-8. PMID 20368646
9. Committee on Cancer Clinical Trials and the NCI Cooperative Group Program; Institute of Medicine. A National Cancer Clinical Trials System for the 21st Century: Reinvigorating the NCI Cooperative Group Program. Washington, DC: National Academies Press; 2010 [updated 2010; cited]; Available from: http://www.nap.edu/catalog.php?record_id=12879.
10. Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2000 Oct 13;103(2):211-25. PMID 11057895
11. Hubbard SR, Till JH. Protein tyrosine kinase structure and function. Annu Rev Biochem. 2000;69:373-98. PMID 10966463
12. Skolnik JM, Adamson PC. Tyrosine Kinase Inhibitors in Pediatric Malignancies. Cancer Invest. 2007 Oct 18:1-7. PMID 17952738

The Voice of the Invisible - the experiences and consequences of having a brother or sister with cancer during childhood

Ulrika Kreicbergs

In pediatric cancer, for obvious reasons, the focus is on the sick child. However, over the last few decades the impact on the whole family has been increasingly recognized. Yet, this awareness has almost exclusively been confined to the parents, while the siblings have received little attention. The term invisible sibling has been coined to describe the consequences of having a brother or sister with cancer. Despite their bewilderment, stress and fear, the siblings receive even less attention than normally because the critical priority is their sick brother or sister. Both parents and healthcare professionals are guilty of this understandable omission, and although it might be difficult to widen their focus to the siblings, this may prove to be of decisive importance for the long term recovery of the family as a whole. Notably, the SIOP guidelines emphasize this whole-family approach to the management of children with cancer.(1-3) The scope of this task is daunting when one considers the number of pediatric cancer cases. In United States more than 248 000 children are living with cancer and the incidence of pediatric cancer is increasing.(4) In the European Union approximately 20 000 young people (0-19 years of age) were diagnosed with cancer last year.(5)

The prognosis for children with cancer has improved over the last decades to a cure rate close to 80% in the developed world. However, this fact does not alleviate the strain on the families during the child's treatment, not even after cure has been reassured by the oncologist. In many cases, the fear of recurrence remains for some time to come. In addition, persistent side effects may be a constant reminder of the trauma imposed by the cancer and its treatment. In families losing a child to cancer, the bereavement commonly affects both parents and siblings for the rest of their lives.

Although, there are a number of studies on the experiences of families having a child with cancer, information on siblings is limited, and in particular on those bereaved. In fact, much remains to be known regarding the psychological, social and educational outcomes of siblings of children with cancer. Many questions remain unanswered, e.g.: which are the most stressful moments; what factors in daily life are the most challenging for the siblings; what are the effects on their relationships within and outside the family; what symptoms are elicited by the situation; what special needs in conjunction with the disease of their brother or sister and its treatment do siblings themselves perceive; what could be done to mitigate their stress and suffering; and what measures should be taken by parents and health care staff?

In a review of the literature on siblings of a brother or sister with cancer, Wilkins & Woodgate (6) concluded that siblings have many unmet needs. They perceive their life as changed, both within and outside the family. They sense that the previous family dynamic has been disturbed, and feel separated from their parents. In some cases it has been described that siblings lose their sense of self during this traumatic period. They commonly experience intense feelings of anger, guilt, jealousy, sadness and anxiety. Positive feelings, such as empathy, can also emerge. In their review, Wilkins & Woodgate emphasized that siblings usually seek open communication and involvement in the care of the sick brother or sister, but also want to be supported in their efforts to continue their own interests and activities. They want to feel recognized.

In a study of siblings of a brother or sister newly diagnosed with cancer, Lahteenmaki et.al (7) analyzed the risk of behavioral and psychosomatic problems among the siblings,

and whether that risk decreased over time. Age was also considered in the analysis. Among older, school-aged siblings, learning, psychosomatic and behavioral problems were reported by the parents. In siblings below school age, behavioral and psychosomatic problems, also assessed by their parents, did occur but decreased over time. Thus, even young siblings were found to express feelings of jealousy, envy and loneliness. Most often it seems taken for granted that the young siblings' needs will be met within the family, and therefore they may be overlooked by health care professionals.(8)

Houtzager et al. (9) studied family functioning following a pediatric cancer diagnosis and found that siblings are most distressed during the first months following the diagnosis. Older siblings, especially girls, are at increased risk of psychosocial problems. Alderfer et al. (10) had reported similar results in an earlier study. Indeed, childhood cancer appears to be associated with considerable difficulties in keeping the family together and in taking care of each other. In particular, siblings' sense of self is often shaken by the diagnosis and treatment of their brother or sister. Yet, it remains to be determined whether this loss of self is reversible or not. On the whole, data on the long term effects on siblings is very sparse. In this context, it would be of considerable interest to assess whether there is a significant difference in outcome between bereaved and non-bereaved siblings.

Interviews of bereaved parents suggest that siblings suffer a great deal following the loss of a brother or sister to cancer.(11) Suicide attempts and repeated accidents have been reported among bereaved siblings, although the relationship between these events and the bereavement has yet to be explored. Bereaved siblings have had to endure the protracted illness of their brother or sister as well as the end-of-life period and, finally, the loss. All these experiences are bound to have a negative effect on the siblings' long-term psychological health. According to interviews by Nolbris & Hellstrom (12) siblings were dissatisfied with the information given to them and felt that they had not been involved in their brother or sister's dying process. They expressed loneliness and the need to mourn in their own way by randomly over time entering and exiting their grieving process. In a review by Giovanola (13) on siblings' involvement at the end-of-life, the author examined the sibling relationships, children's perception of death, grief and bereavement. Findings revealed that health care professionals often fail to recognize siblings' needs at end-of-life, while parents attempt to protect the siblings from involvement at end-of-life. In both cases there is a failure to meet the siblings' needs. They are left out and remain "invisible".

Most studies on siblings with a brother or sister with cancer have been exploratory and descriptive in nature. Although a few quantitative studies have been done on non-bereaved siblings, none has been conducted on the long term effects of loss of a brother or sister. Such studies are sensitive by nature, and thus, challenging to carry out, raising ethical concerns about reopening old wounds. Yet, such concerns may be unfounded. Kreicbergs et al encountered hesitation among professionals within pediatric oncology as well as ethical boards about the appropriateness of approaching parents who had lost a child to cancer several years earlier. However, after IRB approval and completion of the study it was found that the vast majority of the parents perceived the follow up as valuable and were positively affected by their participation. (14) This would seem to suggest that also bereaved siblings would not mind being approached for possible participation in a similar study in attempts to identify health care related factors that can be avoided or modified. Findings from such a study could be helpful in designing strategies to prevent or mitigate sibling's suffering and reduce the psychological morbidity in the long term perspective.

Health care professionals have an important role not only in supporting parents, but also in encouraging them to involve the siblings in their brother or sisters' illness from diagnosis to treatment and for some even to the unavoidable death.

References

1. Spinetta JJ, Jankovic M, Masera G, Ablin AR, Barr RD, Ben Arush MW et al. Optimal care for the child with cancer: A summary statement from the SIOP Working Committee on Psychosocial Issues in Pediatric Oncology. Pediatr Blood Cancer 2009; 52 (7):904-907.

2. Spinetta JJ, Jankovic M, Eden T, Green D, Martins AG, Wandzura C et al. Guidelines for assistance to siblings of children with cancer: report of the SIOP Working Committee on Psychosocial Issues in Pediatric Oncology. Med Pediatr Oncol 1999; 33 (4):395-398.

3. Masera G, Spinetta JJ, D'Angio GJ, Green DM, Marky I, Jankovic M et al. SIOP Working Committee on Psychosocial Issues in Pediatric Oncology. Med Pediatr Oncol 1993; 21 (9):627-628.

4. Foster TL, Gilmer MJ, Davies B, Barrera M, Fairclough D, Vannatta K et al. Bereaved parents' and siblings' reports of legacies created by children with cancer. J Pediatr Oncol Nurs 2009; 26 (6):369-376.

5. Ladenstein R. Paediatric oncology in Europe: Current situation and future directions. memo - Magazine of European Medical Oncology 2009; 2 (Volume 2, Number 4 /December, 2009):199-200.

6. Wilkins KL, Woodgate RL. A review of qualitative research on the childhood cancer experience from the perspective of siblings: a need to give them a voice. J Pediatr Oncol Nurs 2005; 22 (6):305-319.

7. Lahteenmaki PM, Sjoblom J, Korhonen T, Salmi TT. The siblings of childhood cancer patients need early support: a follow up study over the first year. Arch Dis Child 2004; 89 (11):1008-1013.

8. Massimo LM, Wiley TJ. Young siblings of children with cancer deserve care and a personalized approach. Pediatr Blood Cancer 2008; 50 (3):708-710.

9. Houtzager BA, Oort FJ, Hoekstra-Weebers JE, Caron HN, Grootenhuis MA, Last BF. Coping and family functioning predict longitudinal psychological adaptation of siblings of childhood cancer patients. J Pediatr Psychol 2004; 29 (8):591-605.

10. Alderfer MA, Labay LE, Kazak AE. Brief report: does posttraumatic stress apply to siblings of childhood cancer survivors? J Pediatr Psychol 2003; 28(4):281-286.

11. Kreicbergs U. To lose a child to cancer: a nationwide study of parental experiences. Karolinska Institutet, Stockholm, 2004.

12. Nolbris M, Hellstrom AL. Siblings' needs and issues when a brother or sister dies of cancer. J Pediatr Oncol Nurs 2005; 22 (4):227-233.

13. Giovanola J. Sibling involvement at the end of life. J Pediatr Oncol Nurs 2005; 22 (4):222-226.

14. Kreicbergs U, Valdimarsdottir U, Steineck G, Henter JI. A population-based nationwide study of parents' perceptions of a questionnaire on their child's death due to cancer. Lancet 2004; 364 (9436):787-789.

The Childhood Cancer Survivor Study: Defining Risks among Long-term Survivors

Leslie L. Robison

Abstract

Survival for childhood cancer has increased dramatically over the past 40 years with five-year survival rates now approaching 80%. For many diagnostic groups, rapid increases in survival began in the 1970s with the broader introduction of multi-modality approaches, often including combination chemotherapy with or without radiation therapy. With this increase in rates of survivorship has come the recognition that survivors are at risk for adverse health and quality of life outcomes, with risk being influenced by host-, disease-and treatment-related factors. In 1994, the U.S. National Cancer Institute funded the Childhood Cancer Survivor Study, a multi-institutional research initiative designed to establish a large and extensively characterized cohort of over 14, 000 five-year survivors of childhood and adolescent cancer diagnosed between 1970 and 1986. This ongoing study, which reflects the single most comprehensive body of information ever assembled on childhood and adolescent cancer survivors, provides a dynamic framework and resource to investigate current and future questions about childhood cancer survivors.

It is well recognized that survival rates for many of the childhood and adolescent cancers have improved at a remarkable pace over the past four decades. Today, cure is the likely outcome for most children diagnosed with cancer. Improvements in therapy have increased the five-year relative survival rate from less than 30% in 1960 to 79% in 2004.[1-3] Long-term survival rates vary substantially according to initial diagnosis, demographic characteristics (e.g., age, sex, race), and presenting clinical characteristics (e.g., extent of disease, location, morphology, biologic features). Thus, more recent clinical trials are often designed with the general philosophy of intensifying therapy among patients with a poor prognosis in an attempt to further increase survival while reducing/modifying therapy in patients with a good prognosis to decrease the potential for acute and long-term toxicities without compromising survival.

With the successes achieved comes the need and responsibility to consider the long-term morbidity and mortality associated with treatments responsible for the increases in survival. To varying degrees, it has been shown that long-term survivors are at risk of developing a spectrum of adverse outcomes including early death, second neoplasms, organ dysfunction (e.g., cardiac, pulmonary, gonadal), impaired growth and development, decreased fertility, impaired cognitive function, difficulties obtaining employment and insurance, and overall reduction in quality of life [4-7]. Because of the young age of childhood cancer survivors, and thus the potential longevity of survivorship, the delayed consequences of therapy will likely have a substantial impact on their lives, their families, and on society at large.

Single institution investigations provided many of the initial observations on selected sequelae occurring at relatively high frequencies or associated with severe morbidity. However, many of these single institution investigations and limited consortia are restricted by a small sample size, incomplete patient follow-up, and are often derived from patient populations that are treated on a single uniform protocol. Thus, precise quantification of a complete range of possible adverse outcomes is often impossible. Some studies of long-term survivors have been carried out within established cooperative clinical trials groups, but with varied success. The pediatric cooperative groups have a primary objective of conducting therapeutic clinical trials and, while questions of health-related outcomes

are of interest, the resources do not always exist to provide the necessary support to successfully conduct such non-therapeutic studies. By the mid-1980s it became increasingly clear that there were serious limitations inherent in these approaches, such as small study sizes and incomplete population characterizations, and limited length of follow-up. To overcome these limitations, a consortium of institutions proposed the Childhood Cancer Survivor Study (CCSS) which in 1994 was funded by the National Cancer Institute.

Details concerning the initial establishment of the CCSS cohort, including characteristics of the survivor and sibling cohorts, have been previously published [8]. Briefly, the CCSS cohort is restricted to five-year survivors of the following diagnoses: leukemia, central nervous system cancers, Hodgkin's lymphoma, non-Hodgkin's lymphoma, Wilms' (kidney) tumor, neuroblastoma, soft-tissue sarcoma, or bone tumor. The original CCSS proposal did not restrict eligibility based upon type of malignancy, but funding restrictions limited inclusion to only the most common diagnoses among cancer patients diagnosed before the age of 21 years. Eligibility for entry into the cohort required that a patient be diagnosed between January 1, 1970 and December 31, 1986 and have survived five years from their date of diagnosis (regardless of disease or treatment status). The institutional review board at each participating center reviewed and approved the CCSS protocol and all study participants provided informed consent. Of the 20, 720 eligible survivors identified, 14.6% were deemed to be lost to follow-up after extensive tracing efforts failed to locate them. Of those successfully contacted, 81.2% completed a 24-page baseline questionnaire. The demographic-, disease-, and treatment-related characteristics of participants, contacted non-participants, and those lost to follow-up were compared to determine the potential for bias [8, 9]. To provide a comparison population, a cohort of siblings of survivors was constructed. A randomly selected subset of survivors was asked to identify all their living siblings, from which the sibling closest in age to the survivor was selected and asked to participate. Of the 4782 eligible siblings, 80.4% participated. Information collected from the sibling cohort is, with the exception of cancer-specific topics, identical to that obtained on the survivor population.

There have been four follow-up surveys conducted since the collection of the baseline data from the study cohort. All study surveys are available on the CCSS website at http://ccss.stjude.org. While the specific content of follow-up surveys has varied, each typically updates major health events in addition to collecting information on focused topics (e.g., health utilization, quality of life measures, health behaviors, medical outcomes, mental health, psychosocial outcomes, use of complementary and alternative therapies, etc.). Beyond the bi-annual follow-up surveys, a variety of topic-specific surveys were conducted within the cohort. The majority of these ancillary studies were supported by investigator-initiated grants addressing specific study populations to conduct more in-depth evaluations. Topics of ancillary studies included barriers to healthcare utilization among survivors, psychosexual function among female and male survivors, health behaviors and quality of life among adolescent survivors, prevalence and risk factors for sleep disorders and fatigue, physical function and quality of life among survivors of lower extremity bone tumors, health information seeking behaviors and breast cancer screening practices among female survivors.

To further enhance the scope of research that can be conducted within the CCSS, a biologic repository was established for banking of genomic DNA obtained from buccal cell samples of survivors and siblings, plus peripheral blood samples from survivors with a second or subsequent neoplasm. Lymphoblastoid cell lines were established from the peripheral blood samples. Those study participants who provided a biologic sample have given informed consent for the collection, storage and future utilization of the material to investigate a spectrum of genetic issues including phase I and II enzymes, DNA repair genes and other metabolic pathways. Use of the material to investigate genes known to be associated with disease-risk (e.g., *p53, BRCA, ATM*, etc.) require independent informed consent by study participants. The initial collection of buccal cell DNA utilized a mouthwash-based approach. Currently, the

active cohort members are being contacted and asked to provide a saliva sample using an approach that provides a higher quality and quantity of DNA. CCSS investigators at the Biopathology Center have initiated collection and storage of pathology specimens on second and subsequent malignancies. The inventory of available biospecimens is on the CCSS website.

With the passage of time, the aging CCSS cohort increasingly brings new information about the very long-term effects of treatment. While this addresses an important aspect of cancer survivorship (i.e., impact of aging among patients exposed during childhood), the treatment-related characteristics of the cohort increasingly reflects a greater historical perspective. To maintain the scientific impact of the CCSS resource, efforts are underway to expand the existing cohort by adding five-year survivors diagnosed and treated between January 1, 1987 and December 31, 1999. Expansion of the CCSS cohort through recruitment of individuals who achieve long-term survival after more contemporary treatment is important to improve our understanding about evolving late complications associated with new agents and modalities. Participating CCSS centers have identified 26, 093 eligible 5-years survivors, of whom 20, 729 were selected for recruitment. Recruitment efforts are underway for expansion of the CCSS cohort.

Another priority for CCSS is a greater emphasis on translation of findings into intervention strategies. A primary focus of CCSS has been to quantify the magnitude of, and risk factors for adverse health and quality of life outcomes. While many of these observations have helped define clinical care recommendations and screening guidelines, it is now essential that this information be applied to cancer control efforts including the development of (1) novel primary risk-adapted interventions for newly diagnosed patients, (2) secondary risk-reducing interventions for long-term survivors at risk of cancer-related morbidity, and (3) risk-based screening guidelines for long-term survivor health care. Each of these aspects of translating CCSS data into intervention strategies will require the development and conduct of clinical trials to test the feasibility and efficacy of the intervention. The CCSS resource represents a strong venue for testing of interventions targeted to long-term survivors.

The CCSS has proven to be a highly used source for data analyses and publications. Since establishing the first complete analytic data set containing baseline questionnaire information, completed medical record abstraction, and validation of second malignancies, investigators have conducted analyses on a wide range of outcomes. A compilation of study findings were recently published in the Journal of Clinical Oncology (Table 2) and a full current listing of publications can be found at the CCSS website.

Summary

Over the past 14 years, the CCSS has significantly expanded our understanding of clinical factors predisposing to cancer-related morbidity and mortality. This accomplishment was made possible by making available information on long-term outcomes in a large, geographically-diverse population that is well characterized in regards to demographics, treatment exposures, and outcomes. Through the education efforts of the CCSS, participants receive semi-annual newsletters summarizing findings of the study and addressing topics of health promotion (available to all interested parties at http://ltfu.stude.org. The health behaviors of long-term survivors may, compared to the general population, have a greater impact on the quality and length of their lives. For the pediatric treatment team, including surgeons, radiation oncologists, and oncologists, knowledge of the late effects of therapy is critical for choosing initial therapy for current and future patients, as well as recommendations for appropriate follow-up and screening of survivors. For health care providers and planners, CCSS offers the first opportunity to assess in detail the impact of long-term cancer survivorship on the delivery of care as these cancer survivors age. Lastly, the cohort provides a critical framework and resource for epidemiologists and biologists to investigate current and future questions regarding consequences of therapy, genetic association, disease processes and causation, and quality of life.

Table 1. CCSS Consortium Institutions and Responsible Investigators.

Institution	Investigators
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Children's Hospital, Denver, CO	Brian Greffe, MD*
Children's Hospital Los Angeles, CA	Kathy Ruccione, RN, MPH*
Children's Hospital, Oklahoma City, OK	John Mulvihill, MD*‡
Children's Hospital of Orange County, Orange, CA	Leonard Sender, MD
Children's Hospital of Philadelphia, Philadelphia, PA	Jill Ginsberg, MD*, Anna T. Meadows, MD‡
Children's Hospital of Pittsburgh, Pittsburgh, PA	Jean Tersak, MD*
Children's National Medical Center, Washington, DC	Gregory Reaman, MD*, Roger Packer, MD‡
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Table 2. Reviews of Previously Published CCSS Results

The Childhood Cancer Survivor Study: A National Cancer Institute-Supported Resource for Outcome and Intervention Research Leslie L. Robison, Gregory T. Armstrong, John D. Boice, Eric J.Chow, Stella M. Davies, Sarah S. Donaldson, Daniel M. Green, Sue Hammond, Anna T. Meadows, Ann c. Mertens, John J. Mulvihill, Paul C. Nathan, Joseph P. Neglia, Roger J. Packer, Preetha Rajaraman, Charles A. Sklar, Marilyn Stovall, Louise C. Strong, Yutaka Yasui, and Lonnie K. Zeltzer. *J Clin Oncol*, 27:2308-18, 2009.
Pediatric Cancer Survivorship Research: Experience of the Childhood Cancer Survivor Study Wendy M. Leisenring, Ann C. Mertens, Gregory T. Armstrong, Marilyn A. Stovall, Joseph P. Neglia, Jennifer Q. Lanctot, John D. Boice, John A. Whitton, Yutaka Yasui. *J Clin Oncol*. 27:2319-27, 2009.
Late Mortality Among 5-Year Survivors of Childhood Cancer: A Summary From the Childhood Cancer Survivor Study Gregory T. Armstrong, Qi Liu, Yutaka Yasui, Joseph P. Neglia, Wendy Leisenring, Leslie L. Robison, Ann C. Mertens *J Clin Oncol*. 27:2328-38, 2009.
Chronic Disease in the Childhood Cancer Survivor Study Cohort: A Review of Published Findings Lisa Diller, Eric J. Chow, James G. Gurney, Melissa M. Hudson, Nina S. Kadin-Lottick, Toana I. Kawashima, Wendy M. Leisenring, Lillian R. Meacham, Ann C. Mertens, Daniel a. Mulrooney, Kevin C. Oeffinger, Roger J. Packer, Leslie L. Robison, Charles A. Sklar. *J Clin Oncol*. 27:2339-55, 2009.
Health Behaviors, Medical Care, and Interventions to Promote Healthy Living in the Childhood Cancer Survivor Study Cohort Paul C. Nathan, Jennifer S. Ford, Tara O. Henderson, Melissa M. Hudson, Karen M. Emmons, Jacqueline N. Casillas, E. Anne Lown, Kirsten K. Ness, Kevin C. Oeffinger *J Clin Oncol*. 27:2356-62, 2009.
Ovarian Failure and Reproductive Outcomes After Childhood Cancer Treatment: Results From the Childhood Cancer Survivor Study Daniel M. Green, Charles A. Sklar, John D. Boice, John J. Mulvihill, John A. Whitton, Marily Stovall, Yutaka Yasui *J Clin Oncol*. 27:2363-73, 2009.
Physical Performance Limitations in the Childhood Cancer Survivor Study Cohort Kirsten K. Ness, Melissa M. Hudson, Jill P. Ginsberg, Rajaram Nagarajan, Sue C. Kaste, Neyssa Marina, John Whitton, Leslie L. Robison, James G. Gurney *J Clin Oncol*. 27:2374-81, 2009.
Social Outcomes in the Childhood Cancer Survivor Study Cohort James G. Gurney, Kevin R. Krull, Nina Kadan-Lottick, H. Stacy Nicholson, Paul C. Nathan, Brad Zebrack, Jean M. Tersak, Kirsten K. Ness *J Clin Oncol*. 27:2382-89, 2009.
Psychological Status in Childhood Cancer Survivors: A Report From the Childhood Cancer Survivor Study Lonnie K. Zeltzer, Christopher Recklitis, David Buckbinder, Bradley Zebrack, Jacqueline Casillas, Jennie C.I. Tsao, Qian Lu, Kevin Krull *J Clin Oncol*. 27:2390-95, 2009.
High-Risk Populations Identified in Childhood Cancer Survivor Study Investigations: Implications for Risk-Based Surveillance Melissa M. Hudson, Daniel A. Mulrooney, Daniel C. Bowers, Charles A. Sklar, Daniel M. Green, Sarah S. Donaldson, Kevin C. Oeffinger, Joseph P. Neglia, Anna T. Meadows, Leslie L. Robison. *J Clin Oncol*. 27:2396-2414, 2009.

References

1. Ries LAG, Melbert D, Krapcho M, Mariotto A, Miller BA, Feuer EJ, Clegg L, Horner MJ, Howlader N, Eisner MP, Reichman M, Edwards BK (eds). SEER Cancer Statistics Review, 1975-2004, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2004/, based on November 2006 SEER data submission, posted to the SEER web site, 2007
2. Axtell LM, Asire AJ, Myers MH, editors. Cancer patient survival. Report No. 5 DHEW Publ No. (NIH)77-992. Bethesda (MD): National Cancer Institute. 1976
3. Smith MA, Seibel NL, Altekruse SF, Ries LA, Melbert DL, O'Leary M, Smith FO, Reaman GH. Outcomes for children and adolescents with cancer: challenges for the tweny-first century. J Clin Oncol 28:2625-34, 2010
4. Oeffinger KC, Hudson MM: Long-term complications following childhood and adolescent cancer: foundations for providing risk-based health care for survivors. CA Cancer J Clin 54:208-236, 2004
5. Mertens AC, Walls RS, Taylor L, et al: Characteristics of childhood cancer survivors predicted their successful tracing. J Clin Epidemiol 57:933-44, 2004
6. Reulen RC, Winter DL, Frobisher C, et al: Long-term cause-specific mortality among survivors of childhood cancer. JAMA 3034:172-79, 2010
7. Neglia JP, Friedman DL, Yasui Y, et al: Second malignant neoplasms in five-year survivors of childhood cancer: Childhood Cancer Survivor Study. J Natl Cancer Inst 93:618-29, 2001
8. Robison LL, Mertens AC, Boice JD, et al: Study design and cohort characteristics of the Childhood Cancer Survivor Study: A multi-institutional collaborative project. Med Pediatr Oncol 38:229-39, 2002
9. Mertens AC, Walls RS, Taylor L, et al: Characteristics of childhood cancer survivors predicted their successful tracing. J Clin Epidemiol 57:933-44, 2004

Modeling Bone Marrow Failure Syndromes with Induced Pluripotent Stem Cells

Suneet Agarwal & George Q. Daley

Abstract

Genetic mutations associated with blood diseases are being discovered at an unprecedented rate. To develop innovative new treatments, we must determine how these mutations cause defects in hematopoietic development and cell function. The advent of "direct reprogramming" technology allows us to revert a patient's skin or blood cells to an embryonic state, yielding "induced pluripotent stem" (iPS) cells. iPS cells carrying patient mutations can in turn be differentiated into numerous specific tissue types to examine pathogenesis in a developmental context. iPS technology is well suited for modeling several human genetic blood disorders that have been a challenge to study using engineered mice or primary patient cells. Work-to-date creating iPS cells from patients with bone marrow failure syndromes is providing unexpected insights and prospects for therapy.

Introduction

An important challenge we face in this era of robust disease gene discovery is translating that information to understand pathogenesis. Many monogenic human diseases display significant phenotypic variability because they emerge on different genetic backgrounds, in a multicellular context, and over the time course of development. This complexity limits the insights obtained from gain- or loss-of function analyses of individual disease genes. Gene targeting in mice addresses some of these issues, but subtle disease alleles are difficult to engineer and species differences may result in a failure of the murine phenotype to mimic the human disease. Ideally, human mutations should be studied on their native genetic background and in the cell types most severely affected, but procurement of appropriate amounts and types of patient material is difficult. These challenges are particularly true for studying human bone marrow failure syndromes, a rare and heterogeneous group of variably penetrant, congenital disorders which sometimes manifest with multisystem developmental defects. Here we describe the new opportunities that iPS technology affords for understanding, and hopefully one day treating, human bone marrow failure syndromes.

Direct reprogramming and induced pluripotency

iPS technology is rooted in the concepts of nuclear equivalence and cellular plasticity, which have emerged through decades of work in basic developmental biology. Somatic cell nuclear transfer (SCNT, or "cloning") experiments have proven that, despite adopting several different functional identities over the course of development, adult cells retain the potential (in the form of genetic material) to recreate an entire organism. In SCNT, the nucleus of an adult somatic cell is introduced into an enucleated oocyte, whose ooplasm "reprograms" the transferred genome and re-initiates an embryonic gene expression sequence leading to the development of an adult animal. In a landmark report in 2006, Takahashi and Yamanaka reduced much of the daunting complexity of oocyte-mediated reprogramming to a set of defined gene products[1]. In Yamanaka's "direct reprogramming," a combination of transcription factors (e.g. OCT4, SOX2, KLF4, cMYC, NANOG, LIN28) is ectopically expressed in adult somatic cells. Subsequent culture of transduced cells under embryonic stem (ES) cell conditions permits the identification and isolation of cells that have reverted to an embryonic-like state, so-called induced pluripotent stem (iPS) cells. iPS cells share several critical properties with ES cells: (1) the ability to divide and replicate endlessly

(*self-renewal*); (2) amenability to genetic manipulation via homologous recombination; (3) and the ability to give rise to any tissue in the body (*pluripotency*). Early controversy about whether iPS cells fulfill the strictest functional definition of ES cell pluripotency has been settled by tetraploid complementation experiments, whereby skin-derived iPS cells injected into a defective blastocyst are able to support the development of an entire adult mouse[2-4]. iPS cells derived from a variety of human tissues (e.g. skin, hair follicles, blood cells) meet the gold-standard functional criteria of human ES cell pluripotency, namely the ability to form teratomas comprised of tissues from all three germ layers when injected into immunodeficient mice[5-10]. Human iPS technology thus permits the creation of highly tractable and versatile cell lines carrying our patients' genetic lesions, ushering in new possibilities for disease modeling and autologous cellular therapy.

Bone marrow failure syndromes

Although aplastic anemia most frequently presents without a clear cause (i.e. idiopathic), in some cases the family history, age of onset and/or the presence of additional physical abnormalities raise suspicion for an inherited bone marrow failure (BMF) syndrome. Diagnosing patients with this relatively rare group of disorders has important implications for therapy, and understanding pathophysiology will illuminate normal hematopoietic stem cell homeostasis and development. In several respects, human BMF syndromes are ideal for iPS-based modeling. Despite tremendous progress in defining mutations underlying BMF syndromes, the hematopoietic failure in these diseases remains largely unexplained. A common feature of the genetic lesions in human BMF is that they affect fundamental cellular pathways, and most mutations are hypomorphic rather than null (which would probably be non-viable). Modeling BMF syndromes in the mouse is hindered not only by the relative difficulty of engineering hypomorphic mutations but also the frequent lack of phenocopy. For example, mice deficient in FA pathway genes show molecular evidence of a defect in the DNA damage response pathway, i.e. impaired monoubiquitination of Fancd2, and quantitative and qualitative stem cell defects, but do not develop BMF. Mice with homozygous null mutations of telomerase genes survive for several generations before manifesting any significant abnormalities, whereas humans with heterozygous telomerase mutations present with dyskeratosis congenita. Primary patient samples could be used to study pathophysiology *in vitro*, but the disease-carrying cells of interest (such as HSCs or other stem cells) are typically difficult to procure and propagate. Generating patient-specific iPS cells offers an opportunity to overcome many of these difficulties, as exemplified by recent reports using somatic cells from patients with Fanconi anemia and dyskeratosis congenita. Along the way, the reprogramming experiments using these cells are yielding unexpected findings about the induction and maintenance of pluripotency.

Fanconi anemia

The most common BMF syndrome is Fanconi anemia (FA), a multisystem developmental disorder with premature death due mainly to hematologic failure or malignancy. To date, 13 genes have been found to be mutated in FA, and all appear to function in a common pathway regulating DNA repair. Cells from patients with FA display a characteristic hypersensitivity to DNA crosslinking agents such as mitomycin C. FA exemplifies many of the difficulties with studying human genetic diseases in mouse models or primary patient tissues. Athough mice with deletions in *Fanca*, *Fancc*, *Fancg* and *Fancd2* have been generated, and cells from these mice display increased sensitivity to DNA cross-linking agents, none of the mice develop marrow hypoplasia or leukemia. Direct analysis of primary hematopoietic cells from FA patients is restricted by their limited numbers and poor proliferation, which also has hindered progress in gene therapy. With these issues in mind, several groups have attempted to generate iPS cells from the somatic tissues of FA patients. As recently reported by Raya *et al*., however, reprogramming primary fibroblasts from FA patients from a variety of complementation groups has proven extremely difficult[11]. Rather, if the FA lesion is complemented in the cells by introduction of a wild-type transgene, reprogramming efficiency is restored and iPS

cells carrying the endogenous FA mutation can be generated. Raya*, et al.* found that subsequent knockdown of the correcting transgene led to rapid loss of self-renewal. These results suggest, unexpectedly, that an intact FA pathway is required for induction and maintenance of pluripotency. Why might this be? One possibility is that because cells carrying FA mutations exhibit premature senescence, they are unable to sustain sufficient cell divisions to undergo reprogramming and/or they are unable to induce self-renewal mechanisms while transitioning to a pluripotent state. Moreover, conventional reprogramming strategies such as those employed by Raya, *et al*. depend on numerous retroviral or lentiviral integration events. It is possible that disruption of the DNA repair machinery in FA cells precludes resolution of the DNA breaks associated with viral integration, resulting in cell death. Supporting this possibility is a recent report by Mitalipov and colleagues that using non-integrating transgenes for reprogramming permits the generation of FA iPS cells without prior correction of the underlying genetic lesion[12]. These iPS cells retained the characteristic FA-associated hypersensitivity to DNA crosslinking agents. However, in contrast to the results of Raya *et al*., these disease-carrying FA cells can be propagated continuously. Clearly the FA associated lesions hinder reprogramming, but further studies will be required to elucidate the precise role of the FA pathway in induction and maintenance of pluripotency.

Human iPS cells have the potential to be differentiated to any cell lineage in the body. Importantly, Raya *et al*. showed that transgene-corrected FA iPS cells were able to give rise to hematopoietic progenitors by directed differentiation *in vitro*. Given that hematopoietic stem cell failure is a primary cause of death in FA, these studies set the framework for gene correction in patient-identical pluripotent stem cells, followed by derivation of progenitor cells to replace those in the affected lineage. Importantly, it is unclear what reconstitution potential current human iPS-derived hematopoietic progenitors possess. Gene expression profiles of these and other *in vitro* iPS-derived progenitors suggest a primitive or embryonic hematopoietic cell phenotype. At present, generating definitive blood progenitors capable of long-term hematopoietic reconstitution is an area of intense investigation.

Dyskeratosis congenita

Unlike most human somatic cells, which have a limited capacity to divide in culture, iPS cells derived from these cells are immortal and display indefinite self-renewal. A key determinant of replicative life span is telomere length, which diminishes with each cell division and is restored by the enzyme telomerase. Using normal human cells, we found that telomerase is induced and telomere length is increased in iPS cells relative to the fibroblasts from which they are derived[13]. To further investigate the role of telomerase and telomere dynamics in reprogramming, we attempted to reprogram somatic cells from patients with the BMF syndrome dyskeratosis congenita (DC). DC is a rare disorder with multiple inheritance forms, characterized by skin pigmentation changes, oral leukoplakia, and dystrophic nail changes as well as premature degenerative changes in several other tissues. Like FA, BMF and hematologic malignancy are the primary causes of mortality in DC. Elegant work in the past 10 years has defined mutations in 6 genes, all involving the telomerase or telomere complex, which collectively account for approximately 50% of DC cases.

We investigated whether defects in telomerase function would limit derivation or self-renewal of iPS cells from patients with DC. We reprogrammed primary fibroblasts from patients with X-linked and autosomal dominant DC, caused by mutations in the genes encoding dyskerin and telomerase RNA component (*TERC*), respectively. We were able to establish multiple DC-specific iPS lines showing all hallmarks of pluripotency, including the formation of hematopoietic progenitors *in vitro*. Unexpectedly, DC-specific iPS cells were able to sustain continual proliferation *in vitro*, in contrast to the premature senescence displayed by the DC fibroblasts, and we found that telomere length in DC iPS cells increased with continued passage in culture. To explain this finding, we discovered that steady state levels of *TERC*, which are critically limiting in several forms of DC, are upregulated in normal and DC iPS cells. We found that *TERC* upregulation is a

feature of the pluripotent state and that the *TERC* and *DKC1* loci are bound by pluripotency-associated transcription factors. These findings demonstrate that reprogramming restores self-renewal capacity in DC cells despite genetic lesions affecting telomerase. The ability to generate DC iPS cells permits further genetic manipulation (e.g. gene complementation and correction) and provides limitless cells to explore the vast phenotypic variability amongst individuals with telomerase mutations. At the same time, these observations add to a growing literature implicating *TERC* as a limiting factor in multiple inheritance forms of DC, and suggest that strategies to enhance endogenous *TERC* expression should be feasible and therapeutically beneficial in DC patients.

Pearson marrow pancreas syndrome

Unlike other reprogramming methods such as nuclear transfer, direct reprogramming retains the cytoplasmic contents of the target cell and thus provides a unique opportunity to model mitochondrial genetic (mtDNA) disorders. mtDNA mutations are implicated in numerous congenital and degenerative diseases. There are no curative therapies and consequently mutations in mtDNA cause significant morbidity and mortality. In congenital mtDNA disorders, a mixture of normal and mutated mtDNA (termed heteroplasmy) in the oocyte is partitioned randomly in tissues during embryogenesis. The degree and distribution of heteroplasmy in adult tissues determines the severity and marked phenotypic heterogeneity of the disease. Importantly, mtDNA heteroplasmy is not static but fluctuates in stem cells, germ cells, and cancer cells. The mechanisms driving these changes are unknown.

Pearson marrow pancreas syndrome (PS) is a rare BMF syndrome caused by heteroplasmic deletions in mtDNA. The clinical hallmarks of PS include transfusion-dependent sideroblastic anemia and other cytopenias, pancreatic insufficiency, metabolic acidosis and other systemic organ dysfunction. The cause of the hematopoietic failure is unknown, and modeling the disease using engineered mtDNA mutations in mice has been very difficult. From a patient with PS in our clinical practice, we isolated bone marrow-derived fibroblasts carrying the causative mtDNA deletion. To understand the effects of mitochondrial dysfunction on reprogramming and pluripotency and to model sideroblastic anemia *in vitro*, we reprogrammed the PS fibroblasts into iPS cells. Although reprogramming efficiency of PS cells was very low, iPS cells carrying the pathogenic mutation could be generated. We found that PS iPS cells initially displayed extremely slow growth and a propensity for differentiation, but over time, these culture characteristics improved. Remarkably, propagation of the PS iPS lines resulted in a steady decrease of the mutant mtDNA genome as a function of passage. We were subsequently able to generate several iPS subclones with virtually undetectable amounts of mutant mtDNA, but which retained a clonal viral integration fingerprint showing that they were derived from the original highly heteroplasmic iPS line. These results suggest that changes in cellular physiology accompanying reprogramming to the pluripotent state are highly dependent on intact mitochondrial function and provide strong selective pressure to segregate mutant mtDNA. Notably, a similar negative selection against mutant mtDNA can be observed over time *in vivo* in certain tissues (such as hematopoietic cells) of patients with PS and other mtDNA disorders. From “purged” iPS cells, we were able to generate hematopoietic progenitors without any detectable mutant mtDNA, thus yielding genetically-identical, disease-free iPS cells and blood cells from our patient with PS.

This work provides a unique set of cellular models carrying varying degrees of mitochondrial heteroplasmy on an otherwise identical genetic background to study the effects of mitochondrial genetic defects on tissue development *in vitro.* Our preliminary results show that iPS cells carrying a significant burden of mutant mtDNA give rise to sideroblastic erythroid progenitors after *in vitro* differentiation, whereas disease-free iPS cells of the identical genetic background do not. PS iPS cells therefore provide a valuable opportunity to determine the factors driving changes in mtDNA heteroplasmy in stem cells, which holds important therapeutic implications for a variety of congenital and acquired disorders.

Therapeutic potential of iPS cells for BMF

In general, allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative modality for inherited BMF, yet because of multiple organ pathology caused by the underlying mutation, BMF patients typically suffer unacceptable toxicity with conventional transplantation regimens. Graft versus host disease (GVHD) continues to be a significant life-threatening limitation to allogeneic HSCT. There has been considerable interest in gene therapy for BMF diseases because of the expectation that corrected HSCs will possess a functional advantage after transplantation. However, gene therapy is limited by difficulty procuring and expansion sufficient HSCs in the clinical setting of BMF, and by concerns for random integration of gene correction vectors that cause malignancy. In this context, iPS technology offers several advantages: (1) the generation of limitless pluripotent stem cells which can in theory be used to generate progenitors of any desired tissue; (2) the feasibility of targeted genetic correction strategies; (3) an autologous cell source which avoids the significant complications of conditioning and GVHD associated with allogeneic HSCT.

We face several challenges on the road to patient-specific iPS-based cellular therapy. First, the original reprogramming strategies using retroviral transgenes cause insertional mutagenesis, and re-expression of oncogenic factors such as c-MYC cause tumors in iPS-derived mice. Several strategies to circumvent this problem have been reported, including transient or non-integrating DNA expression systems, proteins, RNA and/or small molecules. Reprogramming efficiencies using these methods are poor but improving quickly, and should not pose a significant technical barrier. Second, patient somatic cells and iPS cells should ideally be derived free of animal products or cell lines ("xeno-free") to prevent exposure to pathogens such as latent viruses and prions. Third, gene targeting efficiency is relatively poor in human pluripotent stem cells. Tools such as zinc finger nucleases and adeno-associated viral vectors to increase homologous recombination efficiency, and methods to convert human iPS cells to a more mouse-like pluripotent state, are being pioneered to address this issue. Fourth, and probably the most significant set of obstacles facing the field, is how to derive definitive tissue progenitors of interest from iPS cells, at a scale suitable for human transplantation, and without partially differentiated or undifferentiated cells in the final transfusion/transplantation product. The genetic and epigenetic integrity of the iPS derivatives will also need to be ensured. Finally, although transplantation of iPS-derived hematopoietic stem cells should be straightforward, delivery methods for other derivatives such as neural and cardiac cells will need to be developed.

Summary and future perspective

In the foreseeable future, with the advent of whole exome and eventually whole genome sequencing, we will know the genes underlying many human diseases, and understanding pathogenesis will be the major challenge. Patient-specific iPS cells provide several advantages for exploring genotype/phenotype relationships, in that they: (1) carry naturally-occurring human disease alleles on the patient's genetic background; (2) are self-renewing, yielding limitless disease-carrying cells for biochemical and molecular genetic analysis; (3) can be differentiated to any cellular phenotype, such as embryonic cells or stem cells that may be difficult to obtain; and (4) are amenable to further genetic manipulation for functional analysis and complementation. Translating human iPS technology to cellular therapy faces formidable challenges, but if the history of the reprogramming field is any indication, we may continue to expect the unexpected.

Acknowledgements

GQD is supported by grants from the NIH (RO1-DK70055, RO1-DK59279, UO1-HL100001, and special funds from the ARRA stimulus package- RC2-HL102815), the Roche Foundation for Anemia Research, and Alex's Lemonade Stand. GQD is a recipient of Clinical Scientist Awards in Translational Research from the Burroughs Wellcome Fund and the Leukemia and Lymphoma Society, and is an investigator of the Howard Hughes Medical Institute and the Manton Center for Orphan Disease Research.

Disclosure

GQD is a member of the scientific advisory boards and has financial interest in the following companies: Epizyme, iPierian, Solasia KK, and MPM Capital, LLP.

References

1. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663-76 (2006).
2. Boland, M.J. et al. Adult mice generated from induced pluripotent stem cells. *Nature* **461**, 91-4 (2009).
3. Kang, L., Wang, J., Zhang, Y., Kou, Z. & Gao, S. iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. *Cell Stem Cell* **5**, 135-8 (2009).
4. Zhao, X.Y. et al. iPS cells produce viable mice through tetraploid complementation. *Nature* **461**, 86-90 (2009).
5. Takahashi, K. et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861-72 (2007).
6. Yu, J. et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917-20 (2007).
7. Aasen, T. et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol* **26**, 1276-84 (2008).
8. Lowry, W.E. et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc Natl Acad Sci U S A* **105**, 2883-8 (2008).
9. Park, I.H. et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* **451**, 141-6 (2008).
10. Loh, Y.H. et al. Generation of induced pluripotent stem cells from human blood. *Blood* **113**, 5476-9 (2009).
11. Raya, A. et al. Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. *Nature* **460**, 53-9 (2009).
12. Sritanaudomchai, H. et al. Combination of six factors induce reprogramming of uncomplemented human Fanconi anaemia fibroblasts. in *International Society of Stem Cell Research 8th Annual Meeting* (San Francisco, CA, 2010).
13. Agarwal, S. et al. Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients. *Nature* **464**, 292-6 (2010).

Notes

Notes

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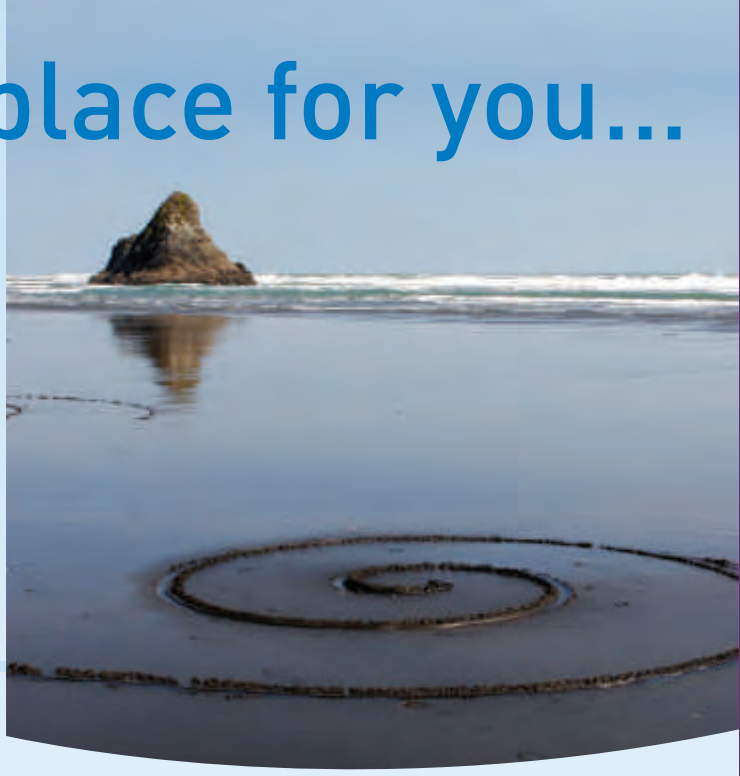

2011 will be a great year to visit New Zealand. We've saved a place for each of you to come and enjoy the science, the companionship and the New Zealand experience.

26-30 October 2011, Auckland, New Zealand

Congress themes:

- Cancer in young adults
- Cancer in indigenous populations

Pre conference activities:

- an education programme for trainees in radiation oncology
- an education programme for nurses
- a symposium on multidisciplinary management of patients with CNS malignancy

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