38th Congress of the International Society of Paediatric Oncology
Geneva, Switzerland · September 17-21, 2006

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Section A

Educational Session: Genetics and pharmacogenetics in pediatric leukaemia

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Section B

Keynote Lectures

Maurizio Arico
Stefan Bielack
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Stefan Meyer
Tom Monclair
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State of the Art Lectures

Henrik Hasle
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Lillian Sung
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Disclaimer

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

CONTENTS

Authors 7

Preface 9

Section A - Educational Session : Genetics and pharmacogenetics in pediatric leukemia

1. **Steroids in ALL**
   Tim Eden 12
2. **How to adapt drug doses in maintenance therapy of ALL**
   Kjeld Schmiegelow 17
3. **Pharmacogenetics and drug dosing in children with Down syndrome**
   Micheal Zwaan 29
4. **Pharmacogenetics in childhood Acute Lymphoblastic Leukemia**
   Ching-Hon Pui 36
5. **From gene expression profiles data to new targeted therapies in ALL**
   Monique den Boer 39
6. **Epigenetic Alterations and targeted chromatin remodeling drugs in pediatric leukemia**
   Max Van Noesel 42
7. **Treatment of MLL gene rearranged ALL**
   Rob Pieters 48
8. **Treatment of Philadelphia positive (Ph+) ALL and CML in children**
   Andrea Biondi 55
9. **New molecular targets for therapy in leukemia**
   Pamela Kearns 59

Section B – Keynote lectures and “State of the Art” Lectures

1. **The new international neuroblastoma Risk Group Staging System - Implications for surgeons**
   Tom Monclair 64
2. **The relevance of nutrition to pediatric oncology**
   Paul Rogers 67
3. **The expanded future of pediatric radiation oncology**
   Sarah Donaldson 71
4. **Hemophagocytic lymphohistiocytosis- A challenge for the pediatric hematologist-oncologist**
   Maurizio Arico 74

CONTENTS

5. **Understanding experiences of childhood cancer: a fresh perspective**
Mary Dixon Woods 77

6. **Pediatric Hematology/Oncology in Morocco**
Mhamed Harif 80

7. **Childhood brain tumours: a developmental disorder**
Paul Scotting 83

8. **Understanding cellular pathways of DNA damage recognition and repair - Clinical implications**
Stefan Meyer 88

9. **Pathogenic mechanisms in Juvenile myelomonocytic leukaemia**
Kevin Shannon 92

10. **Minimally invasive diagnosis and treatment of pediatric malignancies through interventional radiology**
Fred Hoffer 96

11. **Osteosarcoma**
Stefan Bielack 99

"State of the Art" Lectures

1. **Preventing Invasive Bacterial Infection in Neutropenic Patients with Cancer**
Lillian Sung 113

2. **Radiological staging of primary malignant liver tumors in children**
Derek Roebuck 119

3. **Treatment of hepatoblastoma**
Girogio Perilongo, Derek Roebuck, Jean Bernard Otte 122

4. **Treatment of hepatoblastoma - Role of surgery**
Jean Bernard Otte 128

5. **Myelodysplastic Syndrome (MDS) and juvenline myelomonocytic leukaemia (JMML)**
Henrik Hasle 130

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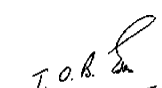
Preface

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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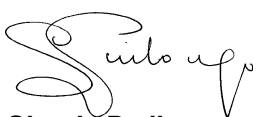
President, SIOP



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SECTION A

Steroids in ALL

Tim Eden

Background

In 1949 Farber first reported that the adrenocorticotrophic hormone (ACTH) which had recently been discovered, could produce short lived remissions in leukaemia and subsequently that cortisone and prednisone had similar activity[1]. After many tentative steps using the then emerging cytotoxic agents for childhood leukaemia, a major breakthrough resulted from concerted activities at St Jude Children's Research Hospital (well reviewed by one of the great pioneers Don Pinkel in 1987[2]). Glucocorticoid induced apoptosis has been incorporated into ALL therapy for the last 3 – 4 decades[3] and in recent times early response to prednisone has been used to predict longterm favourable or adverse outcome in a range of ALL subtypes[4,5]. By the early 1970's the basic template for the treatment of acute lymphoblastic leukaemia had been formulated; firstly induction of remission using daily oral steroids and weekly injections of vincristine over a period of 4 – 6 weeks along with asparaginase injections to deplete lymphoblasts of the essential amino acid asparagine. The need for control of central nervous system (CNS) minimal disease clearly became obvious as remission duration increased[2]. Early injections of intrathecal cytotoxics (Day 1 and repeated during induction) followed by originally craniospinal then cranial irradiation, once remission was achieved, has subsequently been replaced by long courses of intrathecal therapy with or without moderate to high dose systemic methotrexate without loss of efficacy and a marked reduction in long term toxicity. Once remission had been achieved the initial protocols moved on to what became known as maintenance (of remission) therapy but which has subsequently been termed continuation therapy. This phase relied heavily on antimetabolite therapy with oral 6-mercaptopurine and methotrexate. Some trials (particularly in the UK and USA) included 4 – 6 weekly pulses of steroids (5 days) and a vincristine dosage, others just used the antimetabolites[6]. With such basic treatment about 50 – 55% of patients could be cured.

The introduction of post induction intensification/ consolidation modules to further reduce residual disease has led to an improvement in 5 year event free survival to approximately 80% over the last decade. Most such modules also include steroid therapy. Within the context of increasingly sustained intensive therapy and stratification of patients based on initial demographics (white cell count, sex, age) and initial tumour response (response to steroids, blood and marrow clearance and more recently minimal residual disease assessment) it is quite difficult to assess the contribution of individual agents to the treatment success. However in the absence of many, if any, exciting new agents it is imperative that we optimise the dosing and scheduling of the established effective components of treatment

How do steroids induce apoptosis?

Prednisone and Dexamethasone to a large extent mediate their action via a ligand activated transcription factor, termed the glucocorticoid receptor (GR). Clearly for a cell to be sensitive requires adequate receptor expression. What is much less clear are the downstream genes and pathways involved leading to apoptosis or resistance in response to steroids[7,8,9,10]. Understanding these mechanisms is critical if we are to attempt to cure those high risk patients who display steroid resistance. A range of mechanisms have been postulated including the intrinsic apoptotic pathway involving Bcl-2, the extrinsic network involving cell membrane receptors and downstream pathways, release of caspases and disruption of homestasis or intracellular metabolism initiating a death signal[7].

There has been much speculation but little experimental supportive evidence directly linked to clinical cases. Schmidt et al[11] used whole genome expression profiling to identify steroid regulated candidate genes in steroid sensitive children with ALL, as well as cell lines (sensitive and resistant to steroids) and normal lymphocytes. They identified some novel potential candidate genes. These included a glucose metabolic regulator, PFKB2, a transcription factor ZBTB16 and a probable cell cycle regulation protein kinase SNFILK (part of SNF/AMPK family). They have postulated that steroid induced apoptosis in leukaemia results for altered gene expression at the mRNA level and that the previously presumed pathways may not be involved at all. Further studies are in progress to unravel the exact nature of the genes and their functions but the common denominator appears to be disturbance of intracellular glucose metabolism which triggers a "death" signal.

Genome expression profiling has also been used not only to identify genes predictive for poor steroid response, but also molecular response to several cytotoxic agents and cross resistance to differently acting cytotoxics[12,13,14]. Holleman et al have taken 173 children with ALL at initial diagnosis (of whom 145 had B cell precursor ALL and 28 T cell) looking at response to 4 drugs; prednisolone, vincristine, L-asparaginase, and daunorubicin. For the B lineage ALL they identified 124 genes discriminating between resistant and sensitive leukaemic cells of which 121 were "newly" identified. For prednisolone they identified 33 genes with 3 ESTS. In a number of different trials both from Germany, USA and Holland they were able to analyse outcome related to gene expression and to detect a significant relationship with sensitivity. Like Schmidt et al[11] they highlighted transcription factors and metabolic genes as being associated with prednisolone resistance. In subsequent studies not yet in print they have demonstrated increased glycolytic rates in resistant cell lines and lower rates in sensitive cell lines with a potential sensitising effect of 2-Deoxy-glucose reducing prednisolone resistance. One of the other genes identified of great interest is the myeloid cell leukaemia 1 (MCL-1) gene which they found to have elevated expression in resistant cells of the B lineage. This gene is an anti-apoptotic member of the Bcl-2 family and appears to play a role in mitochondrial function. In further work from the Rotterdam group Stam et al (Personal Communication) clearly showed a very similar elevated expression inducing resistance in infant MLL rearranged ALL cells. Furthermore MCL-1 "knock down" sensitises the leukaemic cells to prednisolone. The real value of this interesting work is that it offers potential for more targeted therapy for those showing prednisolone resistance by trying to inhibit high level glucose metabolism. Currently there is development of early phase studies looking at 2-deoxy D-glucose. Alternatively targeting of MCL1 may help although whether the current agents available to do that will prove useful remains to be seen.

Which steroid?

Veerman et al reported a significant improvement in disease free survival compared with a historical comparator when dexamethasone was substituted for prednisolone in the Dutch ALL VI study[15]. This raised considerable interest and triggered two major randomised clinical trials particularly as other groups with superior outcome had empirically used dexamethasone in their intensification modules[5].

In vitro lymphoblastoid cell line cyto toxicity studies comparing the potency of prednisone and dexamethasone showed a 5.5 fold greater effect of dexamethasone in an allogeneic marrow derived stromal support system but a 16.2 fold increased potency for cells cultured without stroma[16,17]. These studies have not really helped us to clarify what the optimal dose of dexamethasone should be or indeed whether higher doses of prednisone might be equi-efficacious. Most therapeutic groups use a prednisone dose of 40mg/m^2 per day but some use up to 60mg/ m^2 per day. Interestingly at the higher dose no clinical difference in outcome was reported between dexamethasone and prednisolone in one Japanese study. Other factors clearly determine dosage decision eg toxicity. Dexamethasone appears to be 17 fold more potent in humans in terms of cortisol suppression[18]. This raises concern about the dosage chosen for ALL trials (the ratio of dexamethasone to prednisolone in the UK ALL 97/99 and CCG studies was 1:6.7) and the risk of toxicity (see below).

Another part of the equation with reference to choice of steroids is the reported greater central nervous system penetration by dexamethasone. Jones et al had earlier reported a reduction in CNS relapses with the use of dexamethasone but did not report on any associated excess toxicity[19]. Baylis et al had demonstrated differences in cerebrospinal fluid penetration of corticosteroids and postulated that this was the reason behind the reduction in meningeal leukaemia[20]. Bostrom et al[21] reported on the results of their CCG 1922 trial in which they compared dexamethasone with prednisone in a randomised fashion as well as daily oral versus intravenous mercaptopurine for standard risk ALL patients, in a 2 by 2 factorial design study. They used doses of dexamethasone 6mg/m^2 per day for 28 days in induction compared with prednisone 40mg/m^2 per day for 28 days. They did include dexamethasone in 5 day pulses throughout continuation therapy and during their delayed intensification modules patients received dexamethasone in a dose of 10mg/m^2 per day for 21 days with tapered reduction post that time. They demonstrated a significant ($p < 0.01$) 6 year reduction in isolated central nervous system relapse rate and a trend towards fewer isolated bone marrow relapses. Their 6 year event free survival was reported at 85% (+ 2%) for dexamethasone and 77% (+ 2%) for prednisone. Again a significant improvement in event free survival. This study overlapped but was not absolutely concurrent with the United Kingdom MRC ALL 97/99 randomised trial[22] where the induction doses used were dexamethasone 6.5mg/m^2 per day in two divided doses for 28 days compared with prednisolone 40mg/m^2 per day in two divided doses for the same duration. They similarly used 5 day pulses during interim maintenance and continuing therapy. Between 1997 and 99 they used their previous MRC short course intensifications and used dexamethasone in the longer third consolidation. From 1999 onwards they had an identical structure to the CCG study. 1603 patients were randomised and like the American study there was a halving of the CNS relapse rate ($p = .0007$) and also an improved event free survival with dexamethasone 84% versus 76% at five years. This was not a trial only for standard risk patients but included all risk categories except infants and mature B cell ALL. Benefit was seen right across the risk groupings.

Questions had previously been raised regarding potential increased toxicity during induction with the use of dexamethasone with particular anxiety about increased infection risks[23] especially when induction therapy included daunomycin[24]. In the CCG 1922 profile there was an identical incidence of bacteraemia during induction in each trial arm (13%) and no significant difference specifically of fungal or viral infections. The group had looked at incidence of febrile neutropenia and duration of hospital stay but there were no differences in early or late stages of therapy with no significant difference between the two arms in terms of infection at any phase of therapy. In the MRC study similarly there was no overall difference in terms of early death (less than 60 days) between those receiving dexamethasone or prednisolone. Both the American and MRC trials looked at other potential steroid related toxicity including behaviour, hypertension, diabetes, myopathy, avascular necrosis, overall osteopenia and any event. In the MRC study there was a significant ($p < .0001$) increase in behavioural change (a 4 fold increased risk) on the dexamethasone arm. The behavioural changes ranged from depression to hypomania but there were no deaths by suicide. All patients who demonstrated abnormal behaviour reverted to normal when dexamethasone was stopped and most were then converted to prednisolone. There was a smaller number of patients with significant behaviour disturbance on prednisolone which improved with reduction in dosage. Myopathy was significantly more often seen in the dexamethasone arm (p value $< .0001$) with a nearly 6 fold increase in proximal limb myopathy which recovered fully with conversion from dexamethasone to prednisone (and with time). Osteopenia which was not so systematically looked at was significantly more commonly reported in the dexamethasone group with a 7 fold increased relative risk (p value $< .05$). No statistical differences were reported for hypertension, diabetes or indeed avascular necrosis. In the CCG study there was a similar excess of steroid induced myopathy with a rate of 4% for the dexamethasone patients and 0.3% for those receiving prednisone. They reported two patients with consistent agitation during their five day courses of dexamethasone and the patients were switched to prednisone. There were four other patients who at parental request,

were changed from dexamethasone to prednisone because of behavioural changes. This seemed to be a lower rate than seen in the UK series. There was not any significant difference in terms of reports of pancreatitis or liver enzyme level elevation between the two arms in their study but there was a higher incidence at 5% (Dexa) compared with 1.5% (Prednisone) for hyperglycaemia. Overall in the MRC study toxicity on the dexamethasone arm was seen across the age range with 16% incidence of any steroid toxicity in the under 2's, 10% in the 2 – 9 year olds and 13% in those older than 10 whereas in the prednisolone arm it increased from 0 in the under 2s, to 3% in the 2 – 9 ages and 15% for those older than 10. This phenomenon of increasing toxicity seen with prednisolone for age, bringing it up to the same level as dexamethasone for older patients has prompted further investigation about the effect of steroid dose in teenagers and young adults. In both these trials the collection prospectively of cases of avascular necrosis was not systematically included but subsequent retrospective review clearly also suggests that the risk is much higher in those trials where older patients are included. In the MRC ALL 97/99 protocol the overall incidence of AVN was 1% but 3.8% for those older than 10 years with no significant difference between those on prednisolone or dexamethasone. Other trials reported a wider range of avascular necrosis depending on the age profile of their patients with a marked increase amongst teenage patients.

More recent studies address some of the issues of longer term toxicities associated with dexamethasone use in particular regarding two aspects, neuropsychological functioning/quality of life, and the effect on bone mineral density. Eiser et al[25] reported using standardised questionnaires for maternal assessment of child health related quality of life and behaviour 3 – 6 months after diagnosis with a follow up one year later. For the 45 families studied there was no significant difference in scores between those receiving dexamethasone and prednisolone. There was clearly recovery with time over the first year of treatment. Although longer term studies need to be performed, this was somewhat reassuring.

van Beek et al[26] looked at bone mineral density, body composition and growth in a cohort of patients with a mean follow up of 12.7 years. 47 received prednisolone, (19 of whom also had CNS irradiation) compared with 43 who had dexamethasone but no CNS irradiation. The overall results showed that the groups did not differ in terms of ultimate height, bone mineral density or body composition on follow up.

We are left with the conclusion that there is excess early toxicity but not mortality associated with dexamethasone but the benefit appears to outweigh that toxicity particularly with the evidence of recovery with time. Further long term studies are required to confirm these preliminary findings. With increasing recognition that teenagers and young adults may benefit from treatment according to childhood type protocols[27, - 32] the effect of age needs to be carefully assessed. Toxicity has been well described by Nachman[33]. In the American series of trials there is a higher incidence of steroid and L-asparaginase induce diabetes mellitus compared with that seen in younger children, an excess of pancreatitis and obviously prolonged steroid administration is associated with an increased risk of avascular necrosis. In the CCG 1882 protocol the incidence of AVN in those over 10 years of age reached 14.2% versus 0.9% for those who were under 10. There was also a sex difference with an excess amongst girls. What the COG group have now done is to give dexamethasone intermittently in order to reduce these risks and also many now consider a capping of the dose to a maximum of 10mg which may be appropriate.

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How to adapt drug doses in maintenance therapy of ALL

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Introduction

6-Mercaptopurine (6MP) and methotrexate (MTX) became pioneering anticancer agents when Farber and Burchenal 50 years ago demonstrated that these drugs could induce morphologic remission in a small proportion of patients with childhood leukaemia[15,28]. In the years to follow the combination of vincristine (VCR) and glucocorticosteroids was found to be more effective in this respect, and even though the short-term response at that time was the primarily goal, durable remissions was achieved for some patients[30,109]. Logically cure came in focus, and through a series of trials using different drug combinations and treatment durations it was demonstrated that the chances of long-term control of childhood acute lymphoblastic leukaemia (ALL) were significantly improved by several years of remission maintenance therapy with a combination of daily oral 6MP at a dosage of 50-90 mg/m^2 and weekly MTX at a dosage of 20-40 mg/m^2 [30,88,109].

Although 6MP/MTX maintenance therapy has been used for almost half a century, the most important antileukaemic effector mechanisms still remain to be revealed. Maintenance therapy could act in several ways including through direct cytotoxic mechanisms[17,121], modulation of apoptotic pathways[31], and changes in stroma support[47,68,69] including by antiangiogenic mechanisms[43,75]. Due to lack of knowledge on how to optimise maintenance therapy and due to the complex patient-dosage-toxicity-physician interaction during the long 6MP/MTX maintenance therapy, there has been a lack of international consensus on how this part of the antileukaemic therapy should be monitored and adjusted[110]. Following a survey among the major ALL collaborative group, the international Ponte di Legno group recently published their recommendations on monitoring and adjustments of childhood ALL 6MP/MTX maintenance therapy[2b]. This review discuss the current rationale for dose adjustments by myelo- and hepatotoxicity and/or pharmacological targets. Other components of maintenance therapy such as intravenous 6MP[76,13], high dose MTX[70], vincristine/glucocorticoid[86] or more intensive reinductions[1], or other drug combinations will not be addressed in this paper.

Is 6-mercaptopurine/methotrexate maintenance therapy really necessary?

Today, most ALL protocols include a 4-6 weeks induction regimen followed by several months of consolidation therapy including CNS directed treatment. Then oral 6MP/MTX maintenance therapy will be given until a total duration of treatment of two to three years, the longer duration being reserved for boys due to their poorer prognosis with shorter therapy[20,34,79,105].

Anecdotal data as well as early studies (when maintenance therapy was very short) have shown that some children with ALL can be cured after only a few months of chemotherapy[63,116]. This is not surprising, since more recent MRD trials have shown that a large fraction of patients have extremely rapid clearance of their leukaemic cell burden with less than 10^{-5}-10^{-6} leukaemic cells in their bone marrow after 4-6 weeks of chemotherapy[71,117]. A duration of maintenance therapy longer than 2-3 years does seem to give a slightly (although significantly) lower risk of relapse, but this may be counteracted by a higher risk of death in remission [1]. But what is then enough? Today, 75-80% of children with ALL can be cured by the intensive treatment protocols offered by most collaborative groups[34,88,105], and consequently the necessity of several years of continuing therapy with oral 6MP/MTX has been quesioned. The German ALL-BFM 81 and 83 randomised trials demonstrated that a reduction of the total duration of therapy from 24 to 18 months

reduced the event-free survival from 83% to 76% ($p<0.05$)[86]. In addition, in the Tokyo L92-13 maintenance therapy study by which all therapy was truncated at 52 weeks, approximately one third of the patients with standard risk (SR) or high risk (HR) ALL developed a relapse, whereas the 5-year event free survival (pEFS) in the very-high risk (VHR) group was similar to historical controls (0.63)[116]. However, the cure rate after relapse was in that study 62% (SR), 41% (HR), and 10% (VHR), respectively, which demonstrates that most of the relapses in the lower-risk groups remained sensitive to therapy. Thus, although a large fraction of patients have a very good chance of cure even with only short maintenance therapy[63,86,116], we so far have lacked the means to identify these patients. Most importantly a long maintenance therapy phase seem especially important for the standard risk patients with the lowest risk of relapse.

Dosage and outcome

The importance of dose intensity was first shown in the 1960'ies in a randomised trial where children with ALL who received maintenance therapy with daily oral 6MP (50 mg/m^2) and weekly MTX (20mg/m^2), VCR (1 mg/m^2), and cyclophosphamide (200 mg/m^2) had longer remission duration than patients who received half-dose of these drugs (chosen to avoid toxicity)[77]. Current ALL protocols have *starting* doses of 6MP and MTX from which dosages should be titrated to obtain a certain degree of myelotoxicity. Due to the large interindividual variations in the pharmacokinetics of 6MP and MTX, patients receiving the same doses per m.sq. will experience very different systemic and intracellular drug exposures[3,4,25,46,49,78,94,103,114,126]. Due to deficiency in thiopurine methyltransferase [123], a few patients will tolerate only very reduced doses of 6MP[2]. On the other hand, patients who are tolerant to standard doses of 6MP and MTX, and for whom maintenance therapy is not intensified, have a poorer outcome compared both with patients who receive a reduced dosage due to leukopenia and with patients who are offered upward dose-adjustments to obtain myelotoxicity[74,73,91,81,92]. Finally, repetitive treatment interruptions due to myelo- or hepatotoxicity are also an adverse factor for outcome[91,81].

Myelotoxicity and outcome

Although several retrospective studies have shown that low white blood cell counts (WBC) and/or low absolute neutrophil counts (ANC) during maintenance therapy are related to a superior outcome[18,33,37,64,92,99,101], physicians may be more willing to decrease the dose of these drugs in case of toxicity than to prescribe upward dose adjustments if the leukocyte counts are above the upper target range[92,74]. Unfortunately, only a single study have randomised patients to have their maintenance therapy adjusted by two different target levels of WBC (1.5-3.0 vs 3.0-4.5 x10^9/L) and in that study no difference in outcome was found[118]. However, the overall event-free survival in that study was rather poor (pEFS 44% at 8 years), the ANC were to be kept above 0.5 x10^9/L in both treatment arms, the WBC levels actually achieved in that study were not registered, and thus it is uncertain whether there really were any difference in the WBC level achieved in the two treatment arms.

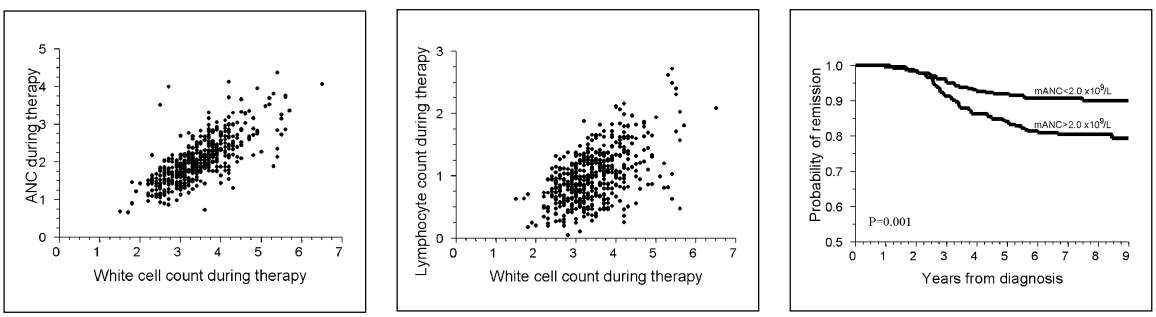

Figure 1a-c: *Myelotoxicity for 538 patients included in the NOPHO ALL-92 trial[92]. For each patient weighted means of neutrophil, lymphocyte, and white blood cell counts during maintenance therapy were calculated. 1c demonstrates the relation between the average ANC during maintenance therapy and probability of continuous remission.*

Both the WBC and the ANC levels are related to the blood cell levels of the cytotoxic metabolites of MTX and 6MP[60,94,103], and both the WBC and the ANC (but not the lymphocyte counts) have been related to outcome[18,23,33,37,64,81,92,99,101], and in all but one of these studies[81], patients with the lower counts had a lower relapse rate. Thus, in one of the most recent studies, patients with an average ANC $<2.0 \times 10^9$/L during maintenance therapy had a 12% lower relapse rate than patients with higher ANC levels[92] **(Figure 1)**. ANC is closely related to the white cell count, and it is therefore uncertain which is superior for dose adjustment (figure 1). The WBC levels during maintenance therapy, however, reflect both the treatment intensity and the patient's normal WBC level. Thus, patients with low WBC levels during therapy also tend to have low WBC after the cessation of therapy ($r_s=0.76$; $p<0.00001$)[100], and even more important, the best predictor of the rise in WBC after cessation of therapy is not the WBC level during maintenance therapy, but the blood levels of the cytotoxic metabolites of 6MP and MTX[104]. This could indicate that a patient with an average WBC during therapy of 3.5×10^9/L would be more intensively treated than a patient with an average WBC of 3.2×10^9/L, if their normal WBC levels were 8.5 and 4.5×10^9/L, respectively[104]. Thus, although the WBC level is a clinically easy parameter by which to target maintenance therapy, this approach has three major weaknesses. First, not knowing the patient's normal WBC level, it is difficult to predict the true treatment intensity for the individual patient. Second, occasionally it is not possible to obtain low WBC levels without unacceptable liver or skin toxicity. Third, an aggressive approach to obtain a high treatment intensity and/or low WBC levels may be counteracted by treatment interruptions or other mechanisms that interferes with the biology of the leukaemic cells[81,92].

Thrombocytopenia is generally not a dose-limiting factor with 6MP/MTX maintenance therapy[104], and as with WBC the thrombocyte count during maintenance therapy is significantly correlated to the thrombocyte count after cessation of therapy ($r_s=0.74$, $p<0.0001$)[104]. Due to hypersplenism, rare patients may experience persistent thrombocytopenia during maintenance therapy, but thrombocytopenia due to veno-occlusive disease, which is frequent with thioguanine therapy, is very rare[90,119].

Hepatotoxicity and outcome

Both 6MP and MTX are hepatotoxic drugs and elevations of serum aminotransferases are frequent during maintenance therapy[8,29,36,96] with rapid normalisation after discontinuation of therapy[8,96]. Although a moderate rise in bilirubin, reduced levels of anticoagulation factors, and hypoglycaemic episodes may be encountered, the risk of serious and/or permanent liver damage seem to be low[35,36]. Carriers of hepatitis B or C virus are at increased risk of hepatotoxicity, but after the introduction of screening procedures for blood products this is a rare cause of hepatotoxicity in countries where the prevalence of carriers of these viruses is low [8,29]. The pharmacological risk factors for 6MP and MTX induced hepatotoxicity have until recently been largely unknown, although a few cases have been related to high erythrocyte levels of the cytotoxic metabolites of 6MP and MTX, i.e. E-6TGN (6-thioguanine nucleotides)[89], methylated 6MP metabolites (E-MeMP)[24], and E-MTX (including MTX polyglutamates)[103], and to accumulation of 6MP in the liver[7]. In accordance with the incidence of hepatotoxicity with methylmercaptopurine riboside therapy[9,39], and the low rate of hepatotoxicity in patients with low thiopurine methyltransferase (TPMT) activity[81,97], we have recently demonstrated that most cases of high aminotransferase levels can be related to high levels of methylated 6MP metabolites[70a97]. The clinical significance of a rise in aminotransferase in current protocols is unclear. Two Nordic studies have shown that patients with a rise in aminotransferases during maintenance therapy had a superior outcome compared to patients without hepatotoxicity [70a,98]. In addition, patients who were kept on therapy in spite of an increase in aminotransferases had a better outcome than patients with treatment interruptions due to hepatotoxicity[91]. A possible explanation for the relation between cure rate and the degree of hepatotoxicity could be the inhibition of the purine *de novo* synthesis caused by MeMP, which may increase the incorporation into DNA of 6-thioguanine nucleotides (the cytotoxic metabolites of 6MP) with subsequent postreplicative DNA mismatch repair[10,11,113,122].

Even with high levels of aminotransferases few patients will have subjective symptoms of hepatotoxicity such as severe nausea, itching, or malaise to such a degree that it requires dose reductions. Most patients with fatigue, nausea, or gastrointestinal complaints during maintenance therapy have normal liver function tests. Consequently, dose reductions in case of high aminotransferase levels are not indicated unless the patient has concurrent objective symptoms such as weight loss, jaundice, or hepatomegali, or biochemical evidence of severe hepatic dysfunction with bilirubin > 50 μmol/L or coagulation factor II, VII, and X < 0.50 U/L. In such cases, other causes such as viral hepatitis or Gilbert syndrome should also be considered[2b].

6-mercaptopurine metabolism and dosage

6MP was developed as a thio-substituted purine analogue to interact with the normal purine metabolism. This innovative drug and a series of similarly effective nucleotide analogues earned Gertrude Elion and George Hitching a well-deserved Nobel prize in 1988[16]. 6MP has three major metabolic pathways **(figure 2)**[54]. First of all it is a prodrug that through a multistep process in part mediated by the enzyme hypoxanthine guanine phophoribosyl transferase is converted into 6TGN that in nucleated cells subsequently are incorporated into DNA[54,113]. A second important metabolic pathway is the breakdown of 6MP to the inactive 6-thiouric acid by the enzyme xanthine oxidase, which is present at high levels in the intestinal tract and in the liver[54,83]. This first-pass effect is responsible for the very variable and generally

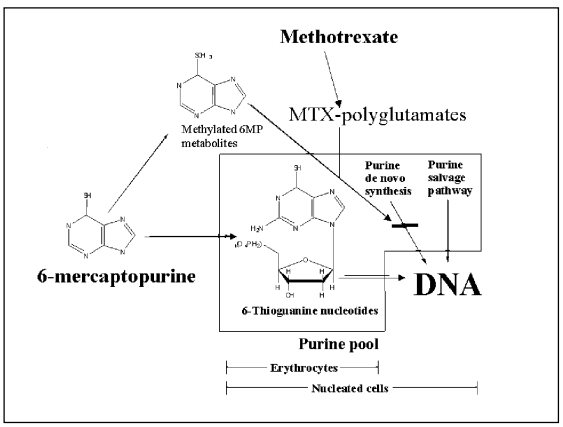

Figure 2: *Simplified diagram of methotrexate and 6-mercaptopurine metabolism and interaction.*

low (16% on average) bioavailability of 6MP [49,126]. Since xanthine oxidase is blocked by allopurinol, the dose of oral 6MP must be reduced when these two drugs are administered concomitantly[125]. The third metabolic pathway is thio-methylation of 6MP and its thioguanine nucleotide metabolites catalysed by the enzyme TPMT[124]. Some of these methylated metabolites, not least methylthioinosine monophosphate, are strong inhibitors of the purine *de novo* synthesis[112]. Previously, MeMP were considered to be largely insignificant for the therapeutic drug monitoring of 6MP/MTX maintenance therapy[54]. However, the purine salvage pathway is very restricted in lymphoblasts that primarily depend on purine *de novo* synthesis (PDNS)[11], and the inhibition of purine *de novo* synthesis could increase the DNA-6TGN incorporation. However, this has not been studied extensively in vivo, and the impact on the cure rate in childhood ALL is largely unknown.

The half-life of an oral dose of 6MP is only 1-2 hours with large inter- and intraindividual variations[48,126]. Even though plasma 6MP concentrations in small studies somewhat surprisingly have been related to outcome [38,44], plasma 6MP measurements cannot be used for treatment adjustments, due to the large intraindividual variations in pharmacokinetics.

Some interindividual variability in the activity of hypoxanthine guanine phosphoribosyl transferase and xanthine oxidase has been demonstrated[56,83]. However, the major determinant of the large interindividual variations in 6MP pharmacokinetics reflects polymorphisms in the TPMT activity[58,82,92,123]. The TPMT activity is routinely measured in erythrocytes where the activity correlates well with that in lymphoblasts[67]. Due to a common polymorphism in practically all studied ethnic groups, 1:300 individuals will be TPMT deficient due to mutations on both alleles, and approximately 5-12% will be TPMT heterozygous [92,123]. Three frequent single nucleotide polymorphisms account for more than 90% of the clinically significant TPMT mutations[72,82]. Compared to wild type patients, TPMT-deficient patients are at risk of severe and even fatal haematopoietic toxicity and will need 6MP dose reduction to approximate 10%, whereas the TPMT heterozygous patients on average will tolerate 85% of the 6MP doses

prescribed to TPMT wild type genotype patients, will experience more treatment interruptions, have twice as high mE-6TGN levels, and experience slightly lower WBC levels during maintenance therapy (figure 4)[58,2,82,92]. Little is known about the cure rate of TPMT-deficient patients, but the heterozygous patients seem to have lower risk of relapse, but also a greater risk of myelosuppression and, more worrisome, second cancers[58,84,85,92,115]. During therapy the antimode of the TPMT activity frequency distribution is 13-14 U/ml red blood cells[82,92], whereas the TPMT heterozygotes have less than 10-11 U/ml red blood cells at the time of diagnosis and off therapy[82] **(figure 3)**. These changes in TPMT activity at different stages of disease probably primarily reflects changes in the age distribution of the red blood cells, since the TPMT enzyme activity is lower in the older erythrocytes[55].

Due to the lack of sufficiently sensitive or reliable assays, measurements of DNA-6TGN in nucleated blood cells have not reached clinical routine for monitoring of 6MP therapy[120,21]. Pioneered by Lynne Lennard and John Lilleyman many research groups have instead measured 6TGN in the non-nucleated erythrocytes, where 6TGN are the end product[12,26,59,82,94]. After a few weeks of oral 6MP therapy a steady state level in E-6TGN is obtained and blood sampling is not dependent on the time point of measurements in the hours after intake of 6MP[27,94]. E-6TGN fulfils most of the demands for therapeutic drug monitoring: **i)** The interindividual variations are much larger than the intraindividual variations, **ii)** after a few weeks of therapy a steady state in E-6TGN is achieved and the level is not affected in the hours after 6MP intake, **iii)** the E-6TGN levels reflect the TPMT-genotype/phenotype, **iv)** E-6TGN is relatively easy to measure although different groups have used different assays, and no standards exist[14,27,53], and most important **v)** E-6TGN levels are related to both myelotoxicity and to risk of relapse[12,62,92,102], although not all groups have been able to confirm this[81]. However, even though E-6TGN in retrospective analyses has been related to the risk of relapse[12,62,92,102], this parameter cannot easily be integrated into dose adjustment guidelines. First, the E-6TGN levels are only surrogates of the fate of 6TGN in nucleated cells, and several studies have indicated that merely securing high cytosol levels of 6TGN may be insufficient to reduce the risk of relapse. Thus, although patients randomised to received 6TG instead of 6MP do achieve very high E-6TGN levels, they do not experience significantly more leukopenia and may not achieve a significant reduction in relapse rate[26,36a,41,51]. Second, patients with low TPMT activity, not least those that are TPMT deficient, also tolerate 2-10 fold higher (red blood cell) cytosol 6TGN levels than patients with a wild-type TPMT geno- and phenotype, which also indicates that cytosol 6TGN concentrations are not directly reflected in the DNA-6TGN levels[2].

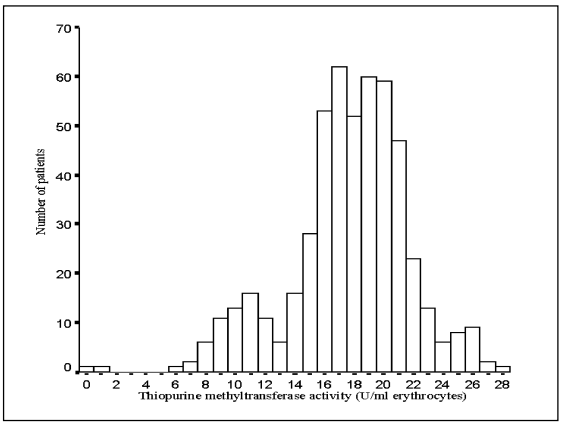

Figure 3: *Frequency distribution of thiopurine methyltransferase activity during 6-merrcaptopurine/methotrexate maintenance therapy (data from NOPHO ALL-92 study, ref 92).*

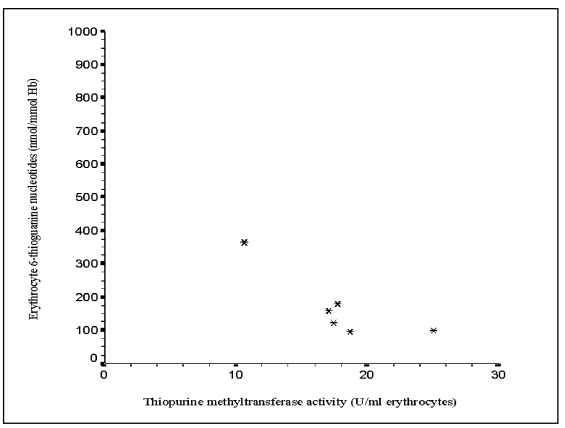

Figure 4: *Scattergram of the relation between weighted means of 6-thioguanine nucleotides in erythrocytes and TPMT activity during 6-merrcaptopurine/methotrexate maintenance therapy data from NOPHO ALL-92 study, ref 92).*

Third, 6MP dose increments to achieve higher E-6TGN levels will preferentially increase the E-MeMP levels[26]. Finally, we recently found an increased risk for a second leukaemia or myelodysplasia both in patients with high E-6TGN levels and in patients with high E-MeMP levels[115]. This observation indicates that methylated 6MP metabolites may influence the risk of DNA damage in some patients possibly secondary to 6TGN incorporation into DNA.

Methotrexate metabolism and dose monitoring

The folate analogue MTX is a prodrug, that similar to natural folates needs to be converted into MTX polyglutamates (with 2-7 glutamyl residues) to exert its effects. Thus, the most important cytotoxic metabolites of MTX are the MTX polyglutamates[17,66]. The propensity for MTX polyglutamation is higher for B-cell precursor (not least the high-hyperdiploid cases) than for T-cell ALL[6,32], which can explain the necessity for higher MTX doses to cure T-cell disease. MTX and its polyglutamates bind tightly to and inhibit dihydrofolate reductase, the enzyme that is responsible for reducing the folates to their active tetrahydrofolate form[17]. The shortage of reduced folate is also determined by the thymidylate synthesis because this oxidizes tetrahydrofolate to its inactive dihydrofolate form. At standard doses, the bioavailability of MTX is good, although it is significantly reduced at oral doses above 40 mg/m^2 [114]. To secure medication compliance and to increase the systemic drug exposure parenteral MTX has been used[2b,19,82], but intramuscular MTX has not been shown to relate to a lower relapse rate than oral MTX[19].

During weekly low dose oral MTX therapy, MTX accumulates in red blood cells until a steady state E-MTX level is achieved after 4-6 weeks[103,106]. The incorporation of MTX in the erythrocytes takes place in the red cell precursors in the bone marrow and MTX with the longest glutamyl residues is retained in the erythrocytes throughout their life span[106,107]. Several aspects of E-MTX have indicated that it could be useful for therapeutic drug monitoring: **i)** The interindividual variations are much larger than the intraindividual variations, **ii)** after 4-6 weeks of therapy a steady state in E-6TGN is achieved (but due to the terminal halflife of 6-8 hours E-MTX should not be measured within 48 hours from the latest intake of oral MTX), **iii)** the E-MTX levels reflect the bone marrow MTX exposure[106], **iv)** E-MTX is relatively easy to measure[42,108], and most important **v)** E-MTX levels have been related to the risk of myelotoxicity[103,104]. However, only one large study have found E-MTX levels to be significantly related to the remission duration[104], and this could not be confirmed in a larger subsequent study, although this in part could reflect the more intensive use of high-dose MTX (HD-MTX) in that study[92].

Methotrexate and 6-mercaptopurine interaction

Several in-vitro and in-vivo studies have shown significant synergistic effects of combined MTX and 6MP therapy. MTX can increase the bioavailability of 6MP through its inhibition of xanthine oxidase **(figure 2)**[4]. Through its inhibition of purine *de novo* synthesis not least for the long-chained MTX polyglutamates, and subsequent increased availability of phophoribosyl pyrophosphate, MTX can increase the conversion of 6MP into 6TGN[10]. Finally, higher E-6TGN levels during 6MP/MTX maintenance therapy correlate with E-MTX levels, although only for the long-chained MTX polyglutamates[22]. The clinically impact of these interactions is uncertain for low-dose MTX/6MP maintenance therapy, but has clearly been demonstrated for combined low-dose oral 6MP and HD-MTX therapy, where the risk of severe myelotoxicity following HD-MTX is significantly decreased when the dose of concurrently given 6MP is reduced, even if the HD-MTX dose is unchanged[70,93]. The most important conclusions of these studies are that dose adjustment of one drug can influence the risk of toxicity induced by the other, and that in case of dose limiting toxicity during maintenance therapy it is probably better to lower the dose of both drugs than stopping one drug and continue the other.

Maintenance therapy and pharmacogenomics

A large number of studies have indicated that single nucleotide polymorphisms (SNPs) in genes that affect the disposition of anticancer agents can influence the outcome of childhood ALL (for review see[2a]). However, apart from TPMT, none have these have so far had impact on the recommendations for dosage or monitoring of MTX/6MP therapy.

Monitoring treatment compliance of maintenance therapy

Several groups have reported poor treatment compliance during maintenance therapy in a few percent of childhood ALL patients and different approaches to address this complication have been proposed[50,52,61,82]. First of all, due to the risk of severe, life-threatening toxicity some collaborative groups recommend that all patients should be phenotyped or genotyped for their TPMT activity prior to initiation of 6MP therapy. In addition, monitoring 6MP/MTX compliance by measuring the levels of 6TGN, MeMP, and MTX polyglutamates has been proposed to detect non-compliers[57,103]. However, routine assessment of these metabolites are expensive and the analyses are only available for routine use in a few centres, luckily at least for 6MP an alternative approach is feasible. Since E-MeMP is strongly correlated to aminotransferases[97] and both E-6TGN and E-MTX levels are related to the degree of myelotoxicity[60,82,94,103], measurements of myelotoxicity and aminotransferase levels could be applied to monitor compliance. Thus, non-compliance should generally be suspected in case of persistent WBC above 3.5-4.0 x10^9/L patients, in spite of prescribed 6MP dose increments above 75 mg/m^2. If the patient by genotype or phenotype is TPMT heterozygous, it is very unlikely that such doses will be tolerated continuously without developing leukopenia[82]. If on the other hand the patient has a TPMT wild type genotype/phenotype, non-compliance should be expected if the patient in spite of dose increments does not experience a rise in aminotransferases.

Circadian schedule

The circadian schedule has been shown to have a striking impact on both the efficacy and toxicity of a number of anticancer agents[40]. In childhood ALL, two 6MP/MTX maintenance therapy studies including more than 400 patients, have shown that the risk of relapse is several fold higher for patients who take their medication in the morning compared to those that are on evening schedule[87,95]. In the larger of these two studies, patients on evening schedule had a 5-year pEFS of 0.82 compared to only 0.57 for patients on morning schedule ($p=0.0002$)[95]. Although it cannot completely be ruled out that compliance to therapy or bioavailability of 6MP and MTX is better with evening administration, and some but not all studies have indicated this to be the case[45,65,52,5], the E-6TGN and E-MTX levels do not differ significantly when patients on morning and evening dosage are compared[95]. Other studies have indicated that differences in the biological activity between malignant lymphoid cells and normal bone marrow cells could determine the chronochemotheraputical findings[80,111]. Whatever the biological mechanism, changing patients from morning to evening is a simple procedure that may have a significant impact on outcome.

Meals and maintenance therapy

In the survey of the MTX/6MP dosage recommendations of the international collaborative ALL groups all groups, but NOPHO, recommend maintenance therapy to be taken without concommittant food especially not milk[2a]. Reduced bioavailability (or even unmeasurable concentrations) have been demonstrated for both MTX and 6MP when the drugs were administered together with food, and it has been recommended that MTX and 6MP should be taken after an overnight fast. The clinical importance of these findings is, however, uncertain: **i)** the number of patients have been <20 in the studies, **ii)** others have failed to confirm the data or found that fasting promotes bioavalability of 6MP only for patients who take >70 mg/m^2/day, and **iii)** titrating the dose of MTX and 6MP by the presence of toxicity should be able to compensate for possible low bioavailability[95]. Finally, restricting the individual mode of drug administration could increase non-compliance. So far only one clinical study have explored the prognostic impact on administration of MTX/6MP maintenance therapy with food, and that study demonstrated no significant difference in outcome depending on whether or not MTX and 6MP were given together with a meal, this being the case also within subgroups defined by whether they took MTX/6MP in the morning or in the evening.

Conclusion

During the last two decades the focus has shifted from standard dosage regimens, to dose titration by myelotoxicity, and more recently to treatment monitoring based of TPMT activity and

erythrocyte 6MP/MTX metabolite measurements. Within a few years direct monitoring of DNA-6TGN in leukocytes will probably also be available. Until it has been determined whether these parameters really improves therapy, we can continue to give these *drugs in the evening*, *adjust the therapy according to the WBC* target guidelines, try to detect and *avoid non-compliance*, and feel humble when the treatment works[2a].

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Pharmacogenetics and drug dosing in children with Down syndrome

Micheal Zwaan

Leukemia in Children with Down Syndrome

Children with Down syndrome (DS) have an increased risk of developing leukemia, which concerns both acute myeloid (AML) as well as acute lymphoblastic leukemia (ALL).[1] DS ALL comprises 1.5-3.2% of children with ALL, and AML in 5-15% (see Table 1 and 2). However, the risk of developing solid tumors appears to be decreased in DS individuals.[1]

Interestingly, children with DS develop distinct biological subtypes of leukemia, when compared with children without DS. As reported by Whitlock et al., children with DS ALL (n=179) less frequently have T-cell disease (7.8 vs. 15.5%, p=0.05) than non-DS ALL (n=8447) patients, and DS ALL does not occur in infants below 1 year of age. Moreover, the cytogenetic abnormalities differ between DS and non-DS ALL: a normal karyotype is found in 45.5% of DS ALL cases vs. 30.3% in non-DS children, high hyperdiploid cases (>50 chromosomes) are rarer in DS (9.1% vs. 25.5%) and no cases of DS ALL with Philadelphia chromosome or t(4;11) have been found.[2] In addition, hypodiploidy and hyperdiploidy (47-50 chromosomes) are more common in DS ALL. The frequency of TEL-AML gene rearrangements is diminished, with 0% in 59 DS ALL patients, versus 19.7% in >2000 non-DS ALL patients.[3]

Similar differences in presentation can be observed for DS AML, where children with DS present at younger age (before the age of 5 years) and with a lower tumor burden than children without DS.[4,5] There may be a pre-leukemic phase characterized by thrombocytopenia and low numbers of blasts. In addition, DS AML differs in morphology, and is usually classified as M0, M6 and, most frequently, M7. It is however questionable whether the FAB classification is useful in DS AML, and Hasle et al. have proposed to classify the disease as a distinct and unique disease entity named 'myeloid leukemia of Down syndrome' (ML DS).[6]

Approximately 10% of children with DS may experience a transient leukemia (TL) in the neonatal period.[7] TL usually does not require treatment, unless complications, such as high white counts, massive organomegaly, effusions or liver fibrosis develop.[8] However, these children need to be followed with 3-monthly complete blood counts for at least 3 years, as DS ML may develop in approximately 20% of children who have been diagnosed with TL.[8] It is currently unknown what causes this transition from a pre-leukemic clone, which is thought to arise in fetal liver hematopoietic progenitors, to a malignant bone marrow disease.[9] Moreover, it is unknown whether children with DS only develop DS ML following TL in the neonatal period, or whether this can also arise 'de novo' without preceding TL. The extra chromosome 21 in DS children is of maternal origin in >95% of children, and there have been reports suggesting that in DS children with TMD or leukemia, the paternal gene is more often involved.[10] Massey et al. showed that children with additional cytogenetic abnormalities at presentation of TL are at greater risk of developing subsequent leukemia.[8]

Considering prognosis, children with DS ALL seem to do worse than their non-DS counterparts, as summarized in Table 1. This is probably not related to steroid resistance, as the BFM-group showed that the frequency of 'steroid good responders' was higher in DS ALL than in non-DS ALL (98.1 vs. 90.7%).[11] It remains questionable whether it is justified to directly compare outcome in DS and non-DS leukemias without taking the genetic differences into account. Especially the lack of hyperdiploidy and TEL-AML1 translocations, which are established good prognostic factors in non-DS ALL, may contribute to differences in prognosis. Several studies point at the poor tolerability for chemotherapy and higher toxic death rates in children with DS ALL (**see Table 1)**. For instance

Table 1: Results of DS ALL studies, compared with non-DS ALL

Study	N DS vs. non-DS	Results in DS patients	Results in non-DS patients	Toxicity in DS	Ref.
BFM	61 vs. 4049 (1.5%)	Good prednisone response 98.1% 6-year pEFS 58% – with therapy reduction 46% (± 13%) – without therapy reduction 65% (± 9%)	Good prednisone response 90.7% 6-year pEFS 70%	Therapy related death 6.6% in DS vs. 1.8% in non-DS ALL	11
CCG 1983-1985	179 vs. 8268 (2.1%)	10-year pOS 68% – standard risk 70%* – high risk 63% Induction failure 2.9%	10-year pOS 77% – standard risk 85% – high risk 65% Induction failure 0.8%	Death in induction 2.9% in DS ALL vs 0.8% in non-DS ALL	2
MRC UK ALL X & XI	55 vs. 3651 (1.5%)	5-years pOS 73%	5-years pOS 82%	Death in remission 11% in DS ALL vs. 2% in non-DS ALL	44
NOPHO 1984-2001	64 vs. 1915 (3.2%)	10-year pOS 57% Induction failure 14%	10-year pOS 80% Induction failure 2%	No excessive toxicity	45

** DS was a significant adverse prognostic factor at multivariate analysis (RR 2.0, p<0.01 for standard, but not for high-risk ALL, classified according to NCI criteria*

in the COG-study, death in induction was 3.6 times as frequent in DS vs. non-DS children, due to GI-bleeding, infection and cardiac failure.[2] Unfortunately, they do not provide data on death in remission, or on complications such as infections or mucositis. Especially methotrexate induced mucositis and aplasia has been described to occur frequently in DS children.[12-14] Higher susceptibility to infections may be due to altered immune function in children with DS.[15]

In the past, many children with DS ML were not offered adequate anti-leukemic treatment, until it was recognized in the early 1990s that children with DS ML were curable when regular chemotherapy was given.[16,17] Further studies showed that cure rates in DS ML were higher than in non-DS AML, and that reduced intensity chemotherapy protocols resulted in better outcome than intensive chemotherapy, as summarized in Table 2.[18] Therefore, children with DS ML should not be eligible for stem-cell transplant in first remission. Some studies showed high toxic death rates, especially in induction **(see Table 2)**.

Recently, somatic mutations in the GATA1 gene, localized to chromosome X, in children with DS TL and ML were documented.[19,20] These mutations appear to be pathognomonic for DS TL and ML, as they were not found in other leukemias (except for rare cases of FAB M7 AML with acquired trisomy 21, and one single adult case with AML M7 without DS or trisomy 21).[20,21] The occasional older child with DS and AML may in fact suffer from sporadic AML rather than GATA1 mutated DS ML.[22] GATA1 is a hematopietic transcription factor involved in normal eyrthro- and megakaryopoiesis. The mutations lead to a shorter GATA1 protein, GATA1s, which may contribute to uncontrolled proliferation of DS ML blasts. This also raised

Table 2: Results of DS ML studies, compared with non-DS AML

Study	N DS vs. non-DS	Results in DS patients	Results in non-DS patients	Toxicity in DS	Ref.
BFM 98	67 vs. 907 (6.9%)	3-year pOS 91% Cum. incidence of relapse 7%	3-year pOS 64% Cum. incidence of relapse 28%	No excessive toxicity	5
CCG 2891	161 vs. 947 (14.5%)	6-year pOS 79% Relapse probability 14%	6-year pOS 43% Relapse probability – standard timing 55% – intensive timing 37%	No excessive toxicity (with standard timing)	4
MRC AML 10 and 12	46 vs. 822 (4.8%)*	5-year pOS 74% Relapse rate 3%	5-year pOS 62% Relapse rate 39%	High early death rate (11%, vs. 4% in non-DS), death in CR 15% vs. 8%.	46
NOPHO 1984-2001	62 vs. 435 (12.9%)	10-year pOS 74% Relapse rate 11%	10-year pOS 53% Relapse rate 41%	No excessive toxicity	45

** Not all children with DS ML were included in this report, hence the true frequency is higher.*

the interest in GATA1 target genes, and their role in leukemogenesis and response to therapy.[23,24] For a more extensive review on GATA1, the reader is referred to a recent review by Crispino.[25]

In-vitro drug resistance studies in DS leukemias

Several groups have studied the in-vitro sensitivity profile of DS leukemic blasts, both in DS ALL and AML. For DS ALL, only limited data are available. Zwaan et al. reported data on 9 successfully tested DS ALL samples, and found no significant differences in in-vitro drug resistance profile compared with non-DS B-cell precursor (BCP) ALL samples, for the drugs frequently used in ALL treatment.[26] However, the control group was unselected, and it is questionable whether that is justified given the skewing of genetic abnormalities in DS ALL when compared with BCP-ALL in general.[2] This may have rendered the control group too sensitive in comparison with the DS ALL samples. Frost et al. compared 5 DS ALL samples with non-DS ALL and found that DS ALL were more resistant to L-asparaginase and dexamethasone, but not to vincristine and doxorubicin.[27]

Considering DS ML, Taub et al. reported that DS myeloblasts were 10-fold more sensitive to cytarabine (Ara-C) than non-DS blasts.[28] They also found approximately 3-fold higher Ara-CTP levels after incubation with 5 micromol/l Ara-C. In a subsequent study, they also reported enhanced sensitivity to daunorubicin (23-fold).[29] Zwaan et al. reported that DS AML cells were significantly more sensitive to cytarabine (median 12 fold), different anthracyclines (2-7 fold), mitoxantrone (9 fold), amsacrine (16 fold), etoposide (20 fold), 6-thioguanine (3 fold), busulfan (5 fold), vincristine (23 fold) and prednisolone (>1.1 fold), than non-DS AML cells.[26] This general hypersensitivity pattern was confirmed by 2 other groups,[27,30] and raises several questions:

1) is this sensitivity due to a general mechanisms (such as an enhanced propensity to apoptosis), rather than to different selective and compound related mechanisms ?
2) as this general hypersensitivity is not found for DS ALL, this suggests that DS ML has unique biological features, which are related to this specific disease and not to DS in general.

Pharmacokinetic data

Pharmacokinetics (PK) is defined as the absorption, distribution, metabolism and excretion of drugs. It is well known that there is substantial (2-10-fold range) inter-individual variability in systemic drug exposure of anti-cancer drugs in children, measured as area under the plasma-concentration curve.[31] This caused by many different factors such as age-related development of in body-composition, renal function, and metabolism. Studies from St Jude Children's Research Hospital have shown that individualized dosing regimens lead to better results when compared with standard dosing based on body surface area (BSA).[32] Most pediatric studies apply specific dosing guidelines for infants (<1 year of age), as they usually have reduced renal and hepatic excretion, reduced metabolism, decreased protein binding, and a larger volume of distribution.[33] Some guidelines for infants have been proposed by Reaman and Bleyer: **a)** decrease cytarabine by 50%, particularly in high-dose regimens; **b)** consider full dose of anthracyclines after 3-6 months of age; **c)** decrease etoposide by 50%, or further in case of jaundice.[34] In the Interfant ALL study, children under 6 months of age get 2/3 of the dose based on BSA, whereas children from 6-12 months of age get 75% of the dose based on BSA.

Children with DS ML have a median age of 1.8 years (range 0.4-16.1 years), hence a reasonable proportion of them will be infants.[4,5] DS children differ in growth characteristics from non-DS children as growth velocity is mainly reduced between 6 months and 3 years of age and during puberty.[35,36] At birth, DS children have heights and weights that are approximately -1 to 1.5 SD when compared with non-DS children. A body weight of 10 kg is usually reached between 1.5-2.0 years of age. Non-DS children have a BSA of 1.0 m2 when they are 30 kg and 140 cm, which is usually achieved around the age of 10 years. However, at that age children with DS have a similar weight of 30 kg, but a height of only 130 cm. Additional growth impairment may arise from cardiac disease, growth hormone deficiency, hypothyroidism and celiac disease.

There are very few studies available regarding PK in children DS. One small study compared 5 children with DS and ALL with 3 non-DS matched

controls, and found almost 2-fold higher methotrexate levels 42 hours after the start of a 1 gram/m2 infusion (0.47 vs 0.24 mumol/L; P = 0.03).[12] This may have been due to the decreased MTX clearance that was observed in these patients. DS children had significantly more side-effects, despite intensified leucovorin rescue in the DS patients. For etoposide, however, this did not appear to be the case, in the 2 DS patients that were studied.[37]

It is therefore difficult to recommend on the issue of dosing in DS children. In the design of a European DS ML study (see below) we asked the participating groups about their guidelines for DS ML. The UK MRC group gave 25% dose-reduction for DS children under 1 year of age, the AML-BFM SG and NOPHO would dose per kg. rather than per BSA, respectively in children under 1 or under 2 year of age. Based on the heights and weights of children with DS ML in the AML-BFM studies, it was calculated that for 1 gram/m^2 cytarabine, dosing based on BSA yielded lower dosages for children below 9 kg, whereas above 9 kg higher dosages were given, when compared to dosing in mg/kg. In the new European DS ML proposal we have decided to use dosing in mg/kg in children of 11 kg body weight or less, which in practice means until the age of 2.5-3.0 years. There is however, a clear need of PK-studies in DS individuals.

Pharmacodynamics

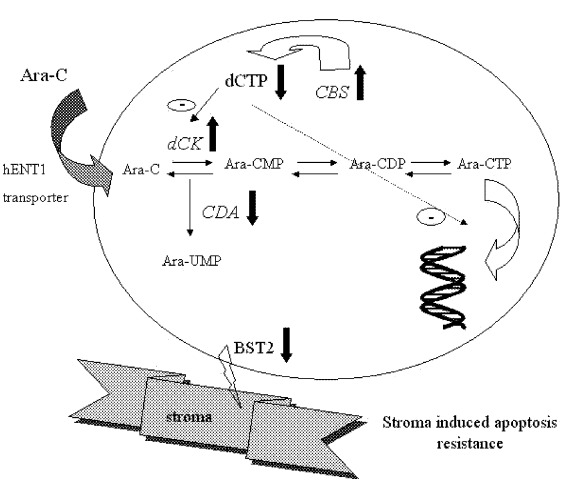
Pharmacodynamics is the relation between pharmacokinetics and pharmacological effect (either toxicity or therapeutic effect) of a given drug in the patient. There are clear differences in pharmacodynamics described for children with DS when compared with children without DS. We will here review the available data for methotrexate, cytarabine and anthracyclines.

Methotrexate

Methotrexate (MTX) is transported into the cell by the reduced folate carrier (RFC), which is localized on chromosome 21q22.[38] This has long been associated with potential greater MTX accumulation, both in leukemic cells as well as in other tissues, such as the gastrointestinal mucosa, explaining the enhanced risk of mucositis experienced by DS patients when using MTX.[13] However, Taub et al. studied the mRNA expression levels of certain genes localized to chromosome 21, but found no increase in RFC-transcript levels, although the number of samples that was studied was very limited.[39] Moreover, when in-vitro MTX resistance was measured as the relative inhibition of the enzyme thymidylate synthase (TS), there were no significant differences between DS and non-DS ALL samples.[26] This also does not suggest

Figure 1: Mechanisms of enhanced cytarabine sensitivity in DS ML cells. *Cytarabine (Ara-C) enters the cell by a nucleoside transporter (hENT1) and/or passive diffusion, after which it gets phosphorylated to its triphosphate metabolite Ara-CTP, which is incorporated into DNA and leads to chain termination and apoptosis. The rate limiting step in the phosphorylation cascade is deoxycytdine kinase (dCK), which is under negative regulation by deoxycytidine triphosphate (dCTP). DCTP also competes with Ara-CTP for incorporation into DNA. Ara-C can be degraded by deamination through cytidine deaminase (CDA).*

DS ML cells are approximately 12-fold more sensitive to cytarabine (Ara-C) than non-DS AML cells, due to several cooperating mechanisms. Cysthationine-â-synthase (CBS) transcript levels are decreased, which indirectly leads to reduced dCTP pools.[41] dCK levels are increased in DS ML.[42] Both CDA and BST2 are potential GATA1 target genes, and are underexpressed in DS AML. BST2 is involved in the interaction between stroma cells and leukemic cells. In stroma cell supported assays downregulation of BST2 resulted in increased Ara-C sensitivity.[24]



enhanced MTX uptake in leukemic cells. This does not exclude tissue-specific effects, and a RFC gene dosage effect could still be responsible for he enhanced frequency and severity of mucositis seen in DS children.[12,13] An alternative hypothesis is that this increased sensitivity is due to pre-existing imbalances in nucleotide pools in DS children, especially due to homocysteine depletion due to a gene-dosage effect of the chromosome 21q22 localized enzyme cysthationine-B synthase (CBS). This enhances folate depletion, and MTX-polyglutamation (polyglutamation of MTX results in increased intra-cellular retention of MTX), and therefore methotrexate sensitivity, although this was debated by other investigators.[14,40]

Cytarabine

The cytarabine (Ara-C) metabolism in children with DS was extensively studied by Taub and Ravindranath et al., as summarized in **Figure 1**. They showed that CBS was 12-fold overexpressed in DS-myeloblasts compared to leukemic cells from non-DS AML patients,[38] which questions the 2:3 expected gene dosage effect with regards to chromosome 21 localized genes. CBS deficiency leads to homocystinuria. CBS overexpression indirectly leads to decreased dCTP pools, and therefore to greater Ara-C triphosphate generation and incorporation into DNA, as was also shown with transfection experiments.[41] dCTP also has a negative regulatory effect on deoxy cytidine kinase (dCK), the rate limiting enzyme involved in cytarabine phosphorylation. dCK transcript levels were reported to be 2.6-fold higher.[42] Hence, the ratio between dCK and CDA favors activation of cytarabine, leading to higher Ara-CTP levels and greater cytotoxicty.

Apart from CBS, Ara-C sensitivity may be influenced by cytidine deaminase (CDA) levels, which is an enzyme involved in the degradation of Ara-C. Transcript levels appeared to be 5-fold lower in DS ML cells, which could be restored by transfection of the wild-type GATA1 gene in a DS ML cell line.[23,42] In a recent study, Ge et al. have compared gene expression profiles of 5 DS ML and 5 non-DS AML FAB M7 samples (which is a relatively small sample set for expression profiling studies and may induce error), and found 551 genes that discriminated between the 2 clusters, which were widely localized on various different chromosomes.[24] One gene, the BST2 (bone marrow stromal cell antigen 2) gene, was underexpressed in DS ML. Evidence was provided that BST2 is a GATA1 target gene, and a transfected DS ML cell line (CMK) was rescued from Ara-C induced apoptosis in a stroma cell assay.

These data provide clear evidence for the enhanced sensitivity of DS ML cells. Children with DS ML have a reasonable tolerance for high-dose Ara-C therapy, and, in line with this, Zwaan et al. did not find differences in drug sensitivity between mononuclear bone marrow cells from healthy children with DS compared with non-DS derived marrow mononuclear cells.[26]

Anthracyclines

The gene encoding for superoxide dismutase (SOD) is localized to chromsome 21q22, and transcript levels in DS myeloblasts were approximately 4-fold higher than in non-DS AML cells.[38] SOD is involved in the generation of oxygen radicals, which may be a mechanism involved in the hypersensitivity of DS ML cells in general, and in hypersensitivity to anthracyclines in particular, as they induce cytotoxicity by free radical formation. Moreover, the conversion of daunorubicin to daunorubicinol is mediated through carbonyl reductase (CBR). The CBR gene is localized on chomsome 21 as well.[29]

Pharmacogenomics

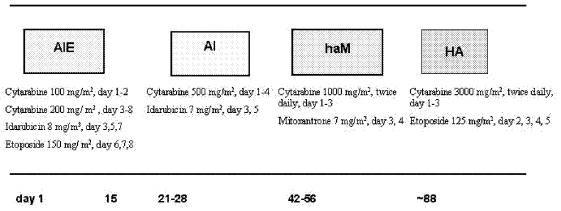
Pharmacogenetics is the study of the inherited basis for interindividual differences in response to chemotherapeutics. A polymorphism has been described in the gene encoding for CBS, i.e. a 68 base pair insertion in exon 8 (844ins68).[43] This polymorphisms was found in approximately 10% in healthy adults, and in 11% of non-DS AML samples. However the frequency was higher (24%) in mononuclear cells of healthy DS children, and even more frequent in DS ML cells (53%). Within the DS ML group, samples with the polymorphisms were approximately 7-fold more sensitive to Ara-C than the group with a WT-allele.

The European DS ML 2006 Study

Recently, a European DS ML protocol was initiated by investigators from Germany, the UK, France and the Netherlands, with Dr. D. Reinhardt from the AML-BFM SG as principal investigator. This study aims at achieving an

overall survival rate of at least 85% for children with DS ML. The protocol was based on the excellent results (3-year pOS of 91%) obtained with the BFM-98 study for children with DS ML.[5] Children with DS ML treated according to this protocol received reduced dosages of anthracylines, and cranial irradiation, HAM-intensification and stem-cell transplant in first CR were omitted. In the new European protocol some further changes have been introduced, for instance no maintenance therapy will be given. The protocol is outlined in **Figure 2**. The cumulative dosage of cytarabine is limited to 27.4 gram/m^2, and anthracyclines to idarubicin 38 mg/m^2 plus mitoxantrone 14 mg/m^2. In the non-DS AML BFM-98 study these dosages were for Ara-C 41-47 g/m^2 (dependent on stratum) and for anthracyclines 64 mg/m^2 idarubicin and 20 mg/m^2 mitoxantrone in arm 1, or 50 mg/m^2 idarubicin and 40 mg/m^2 mitoxantrone in arm 2.

Figure 2: An overview of the European DS ML 2006 study (courtesy of D. Reinhardt, AML-BFM SG, Hannover, Germany). *Outline of the DS ML2006 protocol, which consists of 4 blocks of chemotherapy with reduced dosages of anthracyclines and cytarabine. The protocol is based on the AML-BFM 98 study, with provided separate guidelines for children with DS ML.[5] Please note that the use of this protocol is restricted to study centers, and that not all protocol elements are shown in this graph.*



Conclusions

Children with DS have an increased risk of developing leukemias. DS ML is a biologically distinct and unique disease, and is characterized by good clinical outcome with dose-reduced therapy. This is explained by relative sensitivity to cytarabine and anthracyclines, which has been extensively studies. Data for DS ALL mainly show a different cytogenetic distribution when compared to non-DS ALL. DS ALL patients usually do slightly worse than children with non-DS ALL, which may be the results of enhanced toxicity and therapy resistance. There is a considerable paucity of data regarding drug dosing in DS leukemias, especially with regards to DS ALL.

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Pharmacogenetics in Childhood Acute Lymphoblastic Leukemia

Ching-Hon Pui

Primary genetic abnormalities of leukemic cells are well recognized to have important prognostic and therapeutic significance.[1] Host factors can also influence treatment efficacy.[2,3] For example, given the same dosages of mercaptopurine or methotrexate, reduced accumulation of active metabolites in leukemia cells, due to fast clearance, inactivation, or other reasons, has been associated with a poor outcome in acute lymphoblastic leukemia (ALL).[4] The concomitant administration of certain anticonvulsants (e.g., phenytoin, phenobarbital, or carbamazepine) increases the systemic clearance of antileukemic agents by inducing the production of cytochrome P-450 enzymes, and therefore leads to poor treatment outcome of ALL.[5] Conversely, azole antifungal agents inhibit these enzymes, thereby increasing the efficacy or toxicity of chemotherapy such as vincristine.[1]

Germline polymorphisms in genes that encode for the proteins involved in the pharmacodynamics of antileukemic agents are common, with the frequency of the "variant" allele ranging from 5% to 50%. Alterations of the activity or function of drug-metabolizing enzymes, transporters, receptors, and drug targets result in wide differences among patients in terms of drug disposition and pharmacologic effects, and thereby influence the efficacy or toxicity (or both) of antileukemic agents.[6,7] Traditionally, pharmacogenetic studies have focused on single gene candidates, based on the pharmacokinetic characteristics of a specific drug. Increasingly, a broader strategy, also termed pharmacogenomics, is used to identify the entire set of genes that are relevant to the pharmacological effects of a given drug.[7]

In ALL, the best-studied example of pharmacogenetics is the relation between polymorphisms in thiopurine methyltransferase (TPMT) which catalyses the S-methylation (inactivation) of mercaptopurine and other thiopurines, and the clinical response. Patients who inherit homozygous deficiency of this enzyme require dose reduction of mercaptopurine by 90% to 95% to avoid severe hematopoietic toxicity.[7] Patients with heterozygous deficiency also have a significantly increased risk for hematopoietic toxicity,[8] but they tend to have a better treatment response than do those without this inherited deficiency, possibly because they receive a higher dose intensity of mercapotpurine.[9] In fact, the *TPMT* genotype was linked to early response in a study as assessed by minimal residual disease level on day 78 of remission induction which included mercaptopurine treatment for only 4 weeks.[10] Patients with wild-type alleles were 2.9 time more likely to have positive minimal residual disease than those with heterozygous genotype. Notably, TPMT deficiency has also been linked to a higher risk of second malignancies in patients with ALL, including therapy-related acute myeloid leukemia,[11,12] and radiation-induced brain tumors.[13]

Isoenzymes of the glutathione S-transferase (GST) superfamily inactivate many electrophilic endogenous substances and xenobiotics, including many antileukemic drugs such as anthracyclines, topoisomerase II inhibitors, cyclophosphamide, by conjugating them to glutathione. Although they generally detoxify reactive metabolites, they also play a role in forming cytotoxic metabolites.[14,15] The null genotype of these enzymes has been associated with a favorable response to prednisolone,[16] and to a reduced risk of relapse in children with ALL.[17] A tandem-repeat polymorphism within the enhancer region of the thymidylate synthase gene, one of the major targets of methotrexate, has been linked to increased expression of the enzyme and an increased risk of relapse.[17,18] However, the prognostic importance of these pharmacogenetic variables is treatment-

dependent, and might also be influenced by other genotypes in the context of combination chemotherapy.[19,20]

A number of other pharmacogenetic factors have been associated with outcome. Homozygosity for a polymorphism of methylenetetrahydrofolate reductase was associated with an increased risk of oral, gastrointestinal, or hepatic adverse effects after low-dose methotrexate,[21,22] and with greater in vitro sensitivity of leukemic blasts to methotrexate.[23] The vitamin D receptor Fok I start site CC genotype and thymidylate synthase low activity 2/2 enhancer repeat genotype were associated with an increased risk of osteonecrosis.[24]

Global gene expression profiling of leukemic cells and normal tissue can reveal new dimensions of the pathologic features of leukemic cells, identify new targets for anticancer drugs, and disclose previously unrecognized genomic determinants of cancer-drug resistance and host toxicity. Since drug responses are influenced by multiple genes, polygenic studies and models will be increasingly required to fully elucidate the genetic determinants of drug response.[7] In this regard, the recently available oligonucleotide SNP array[25] and the large scale public genetic databases such as the HapMap project will facilitate these investigations. Finally, it should be recognized that acquisition of additional chromosomes in leukemia cells can create discordance between germ-line genotypes and leukemia-cell phenotypes, including pharmacogenomics.[26]

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From gene expression profiles to new targeted therapies in acute lymphoblastic leukemia

Monique L. den Boer

Abstract

Gene expression profiles can be used to identify genetic subclasses in childhood ALL. We demonstrated that in vitro drug resistance to different classes of drugs is related to specific expression patterns of genes. The profile of these resistance-associated genes predicts clinical outcome independent from all other known risk factors. Moreover, the prognostic value has been independently confirmed in a second group of patients treated on different protocols. Gene expression profiling has the advantage that it is a screening technique that may lead to new (unexpected) ideas about genes important for therapy response and, moreover, may point to to new therapeutic targets. Examples are overexpression of genes involved in glycolysis and MCL-1. Targeting these genes sensitized prednisolone resistant ALL cells to the cytotoxic effect of glucocorticoids.

Introduction

Over the past four to five decades improvements in chemotherapeutic regimens for children with acute lymphoblastic leukemia (ALL) have resulted in cure rates of ~80%. In vivo and in vitro resistance to antileukemic agents are associated with a relatively poor prognosis [1]. However, still very little is known about the underlying genetic defects of drug resistance. Classic resistance mechanisms such as overexpression of multidrug resistance proteins MDR-1, MRP-1, MVP/LRP and BCRP do not seem to play a major role in ALL [2]. Two genetic subclasses of ALL, i.e. those involving rearrangement of BCR-ABL and MLL are associated with a poor outcome but account only for a low number of therapy failures in absolute sense. The majority of therapy failures still occurs in the large so-called favorable genetic subgroups such as hyperdiploid ALL and TEL-AML1 rearranged ALL. This illustrates that the current genetic classification of ALL is not sufficient and that the genes that contribute to therapy responses are unknown. New insights in mechanisms of clinical drug resistance are difficult to obtain because we do not know where to look for. Moreover, many yet known drug resistance-associated genes have been identified in highly manipulated cell line models and hence, many of these "candidate" genes have been shown to be less relevant in patients'cancer cells. Although gene expression profiling techniques have the disadvantage that they are just "fishing expeditions" the advantage is that the whole genome or at least a very large part of the genome is screened which may give us new ideas on genes that might be important for therapy failure and new drug targeting in ALL.

Gene expression profiling, drug resistance and outcome

We have determined the in vitro cytotoxicity to 4 important drugs used in ALL treatment, i.e. prednisolone, vincristine, L-asparaginase and daunorubicin in 173 children with newly diagnosed ALL and compared the gene expression profile of in vitro sensitive- and resistant cases [3]. Out of 14.500 probe sets we identified genes differentially expressed in B-lineage ALL cases that were either sensitive or resistant to prednisolone (33 genes), vincristine (40 genes), L-asparaginase (35 genes) and daunorubicin (20 genes). Out of the total of 124 genes, 121 had never been linked to drug resistance before. A gene expression score was made that combined the expression data of genes linked to resistance for our 4 drugs. A high gene expression score appeared to be significantly associated with a poor outcome: the hazard ratio for relapse was 3.0 for patients with a high gene expression resistance score compared to patients with a low score. The prognostic relevance of this drug resistance-

associated gene expression score was independent of all other known risk factors and was confirmed in an independent population of patients treated with an other protocol that included the above-mentioned 4 drugs. The hazard ratio for relapse for patients with a high score in this population was even 11.9.[3]

Using partly the same set of data we also investigated the differential expression of 70 key apoptosis genes between drug sensitive and -resistant ALL cases [4]. No single apoptosis gene was related to resistance to all 4 unrelated drugs. Expression of MCL-1 and DAPK1 were associated with prednisolone resistance whereas BCL2L13, HRK and TNF were associated with resistance to L-asparaginase. Of these 5 genes only BCL2L13 overexpression predicted outcome significantly. This prognostic relevance appeared to be independent from all other known risk factors and was confirmed in an independent second group of patients treated with another protocol.

The above-mentioned studies do not identify the genes that have the strongest predictive value for outcome because the first selection was made on the basis of in vitro drug resistance phenotype. This leads to a selection bias and an underestimation of the prognostic power of gene expression profiling. It has been demonstrated that the different lineages of ALL (B- versus T-lineage) and the genetic subclasses of B-lineage ALL (TEL-AML1, BCR-ABL, hyperdiploid, E2A-PBX1, MLL) have specific gene expression profiles that can be recognize with an accuracy of more than 95%. This suggests that the underlying biology of these subtypes of ALL differs and in conjunction with this also their underlying resistance mechanisms and the gene expression profiles with predictive value for outcome. Indeed we showed that for instance the mechanism of L-Asparaginase differs between TEL-AML1 rearranged ALL and other genetic subtypes of ALL. In the latter the expression of asparagine synthetase was related to L-asparaginase resistance while in TEL-AML1 rearranged ALL this was not the case [5]. This also implies that it might be difficult to identify one single gene that predicts outcome in all subtypes of ALL.

The group of Willman discovered a new gene that was named OPAL1 (outcome predictor in acute leukemia) of which a high expression was predictive of a favorable outcome in childhood ALL [6]. In two independent cohorts of patients including >400 cases we observed that OPAL1 was highly expressed in TEL-AML1 positive ALL, as was also found by Willman and co-workers. However, we could not confirm the independent prognostic relevance of OPAL1 expression in the total group of ALL patients nor in the genetic subgroups such as T-ALL and TEL/AML1 positive or –negative B-lineage ALL [7]. The prognostic relevance of OPAL1 expression seems therefore therapy dependent or it might be difficult to reproduce gene expression findings from one laboratory to another. The fact however that the gene expression profiles of genetic subclasses of ALL are reproducible between different laboratories suggest that the first explanation may be more likely to be true.

Gene expression profiling may lead to new targeted therapy strategies

An advantage of micro-array technologies is that they may lead to new and unexpected insights into the background of drug resistance and may lead to new genes or pathways that may serve as therapeutic targets. An important example is that our array analyses revealed that prednisolone resistance in B-lineage ALL is associated with an overexpression of genes involved in glycolysis, i.e. the glucose transporter 3 (GLUT3) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). In addition, prednisolone resistant cell lines were shown to have an increased glycolytic rate. The glycolysis inhibitor 2-deoxy-D-glucose (2-DG) sensitized prednisolone resistant but not-sensitive cell lines to prednisolone induced cell kill. This effect appeared to be specific for prednisolone and not for other drugs. Targeting the enhanced glycolysis in ALL may therefore be a suitable approach to modulate glucocorticoid resistance in ALL [8].

A second example is the overexpression of MCL-1 in prednisolone resistant ALL cells. This was found not only in common/pre B-ALL but also in MLL gene rearranged infant ALL. Down-regulation of MCL-1 by RNAi sensitized ALL cells to glucocorticoid induced cell kill. Hence, inhibition of MCL-1 also offers an interesting therapeutic strategy in ALL.

Conclusion

Specific gene expression profiles are associated with resistance to different classes of antileukemic drugs. Profiles of genes associated with in vitro drug resistance have independent prognostic value. Analysis of pathways aberrantly expressed in resistant cells may lead to new therapeutic strategies in ALL.

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Epigenetic Alterations and targeted chromatin remodeling drugs in pediatric leukemia

Max M. van Noesel

Introduction

Cancer formation has long been considered the result of progressive genetic alterations such as mutations in tumor suppressor genes and oncogenes, and chromosomal abnormalities. However, cancer is also a disease of altered epigenetic mechanisms. Epigenetic changes include all mechanisms that alter the gene expression pattern without altering the genetic code. Mostly, these mechanisms are involved with the structure and conformation of the chromatin, and directly determine the level of transcriptional activity of genes. In most cancer types specific epigenetic changes occur and impose semi-permanent epigenetic 'marks', similar to genetic changes[1]. The best-known change is DNA hypermethylation of gene promoters and subsequent gene silencing. However, DNA methylation is only one step in chromatin remodeling and transcriptional silencing of genes. Probably equally important in determining the level of transcription are alterations in the histone proteins that package the DNA.

Here, the mechanisms of epigenetic alterations will be explained in relation to their significance in pediatric leukemia. 'Epigenetics' plays an important role in the tumor formation and possibly tumor progression and can increase our understanding of the malignant cells. More importantly, epigenetic changes are semi-permanent which means that they can be turned-around by so-called epigenetic drugs, leading to restoration of the proper gene transcription. The potential role of several compounds will be discussed in relation to the treatment of pediatric leukemia.

Historic overview of epigenetic research in cancer

In the 1980s, it was observed that a substantial proportion of the DNA in cancer genomes was hypomethylated in comparison to normal tissue genomes[2]. This was the first epigenetic abnormality observed in cancer cells and the loss of methylation involved every tumor type studied, both benign and malignant and even in pre-malignant adenomas. Two functional consequences have become evident:

First, hypomethylation increases the transcriptional level of some genes. Examples of normally hypermethylated genes that are hypomethylated and over expressed in cancer include important genes such as cyclin D2 and Maspin in gastric cancer, MN/CA9 in renal-cell carcinoma, S100A4 metastasis-associated gene in colon cancer and HPV16 in cervical cancer (reviewed in [3]). Also viral transcripts have been detected in increasing amounts in hypomethylated cancer genomes and probably originate from the viral DNA sequences in the 'junk DNA' of our genome.

Second, tumor hypomethylation is linked to chromosomal instability[4]. Hypomethylation is particularly seen in pericentromeric regions. Several cancers, such as Wilms' tumor, ovarian and breast cancers frequently contain unbalanced translocations with breakpoint in pericentromeric regions of chromosomes 1 and 16. In Wilms' tumors, translocation t(1;16) produces Loss of Heterozygosity (LOH) for markers on chromosome 16, which are associated with anaplasia. Another example of chromosomal instability in a hypomethylated region is well illustrated by the ICF syndrome (immunodeficiency, chromosomal instability and facial abnormalities). In ICF, a loss-of-function mutation in one of the DNA methylating enzymes DNA methyltransferase 3B (DNMT3B) causes pericentromeric hypomethylation and chromosomal breakage in the same regions[5]. The causative role of hypomethylation of the genome in leukemogenic translocations or chromosomal instability needs to be proven.

In the 1990s the attention shifted to so-called 'regional hypermethylation' and gene silencing, especially of presumed tumor suppressor genes[6]. Regional hypermethylation has become a hallmark of cancer cells. In normal cells the majority of gene promoters contain so-called CpG-islands, regions of dense CpG- and GC-content stretching from 0,2 to 5 kb. For reasons unknown, these islands are normally protected from methylation and remain hypomethylated. However, in cancer cells, CpG-islands of specific genes can loose the protection from methylation and become regionally hypermethylated. This is strongly associated with transcriptional silencing of the gene. An abundance of studies in adult type tumors and to a lesser extend in pediatric malignancies has shown that: **A.** Silenced genes by hypermethylation are present in virtually all cancer types studied. **B.** Tumor specific methylation patterns have been recognized for different cancer types.

After the recognition of regional hypermethylation and silencing of tumor suppressor genes, again the research field shifted gears and started to explore the mechanisms of chromatin remodeling and cancer treatment. Chromatin remodeling involves not only DNA methylation, but also alterations of the histone proteins. These complex interactions between the histone proteins and the DNA determine the transcriptional activity of gene promoters. Both processes can be influenced by chemical compounds or epigenetic drugs and are potential targets for treatment of cancer patients.

Chromatin structure and the 'histone code'

The level of gene transcription is dependent on the attraction and binding of transcription activators and repressors to gene promoters. Their binding is greatly dependent on the structure of the chromatin. Chromatin can be 'open' (euchromatin) and transcriptionally accessible, or 'closed' (heterochromatin) and transcriptionally repressive. Changing from euchromatin towards heterochromatin is a multi step process on the surface of histone proteins and the DNA. Very distinct chemical steps can be recognized:

The histone code

DNA is wound around histone complexes, which contain several histone proteins 1, 2A, 2B, 3. Especially histone 3 (H3) has lysine-rich tails that stick out of the surface. Several amino acids on the lysine tails can be altered by (de-)acetylation, (de)methylation and phosphorylation. These alterations induce conformational changes of the whole histone complex. For instance, *acetylated* lysine-tails 'stick out' more and induce a more widely spaced chromatin conformation, which is open to transcriptional activators. On the opposite, *deacetylation* of lysines will compact the H3 protein and heterochromatin will be formed. The combination of (de-)acetylated, (de-)methylated and phosphorylated residues on the histone tails is called the histone code. The code therefore refers to a conformational state of the chromatin, which is associated with a certain level of transcription. Only a few details of the histone code are known. For instance deacetylation of lysine (K) in position 9 of the H3 protein (H3K9) and H3K14 and the methylation of H3K9 and H3K27 are strongly associated with a repressive state, whereas methylation of H3K4 is an activating modification (reviewed in [7]). Furthermore, it is assumed that many epigenetic marks are encompassed by the histone code, for instance it probably marks regions for imprinted genes in the genome.

DNA methylation

Methylation of DNA involves the replacement of cytosine (C) by 5'-methylcytosine (5mC). For chemical reasons this can only happen to cytosine in the 5' position from a guanine, a so-called 5'-CpG -3' dinucleotide or simply CpG. Replacing C for 5mC induces conformational changes to the chromatin and DNA methylation is strongly associated with heterochromatin and gene silencing[6]. As a result, transcriptionally repressive Methyl Binding Proteins (MBD's) like MeCP2 and MBD2 are attracted, which eventually recruit other enzymes, like histone deacetylases (HDAC)[7]. These HDACs remove acetyl groups from lysine residues of histone H3 and H4. DNA methylation and histone deacetylation, especially of lysine 9 on histone H3 (H3K9) are strongly connected and associated with gene silencing.

Chromatin, open or closed?

From the above one can conclude on a simplified scheme of how chromatin will change from one conformation to the other. Acetylated histones are associated with euchromatin and gene transcription. If the histones become deacetylated, the conformation changes to heterochromatin and transcriptional repression. Especially H3K9 is a strong repressor and associated with methylation of DNA. Although deacetylation itself is associated with gene silencing, the additional role of DNA methylation is important. DNA methylation is considered a semi-permanent lock into the repressor state. Acetylation and deacetylation are considered relatively 'fluent' states that can change from one into the other **(Figure 1)**. Methylation of DNA represses genes long term, even over generations of cell division.

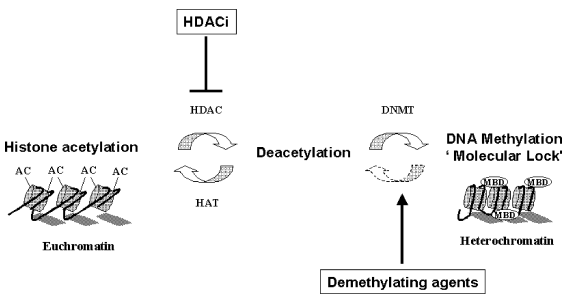

Figure 1: Chromatin remodeling. *Flowchart of interactions between histone acetylation and DNA methylation. Euchromatin can be converted to heterochromatin by histone deacetylation and DNA methylation. Histone acetylation and deacetylation are fluent states. DNA methylation is semi-permanent by lack of any (known) intracellular demethylating enzym. Therefore demethylation can only be induced by demethylating drugs. DNMT: DNA methyltransferases, MBD: Methyl-binding domain proteins, HDAC: histone deacetylases, HAT: histone acetyltransferases, HDACi: HDAC inhibitor*

Epigenetics and leukemia

Comparison of genome wide content of 5mC between normal and neoplastic hematopoietic CD34+ cells has revealed global hypomethylation only in progressive AML in adults and in CML, but not in acute leukemia's ALL[8]. Genome wide demethylation is considered an early event in most cancer types, contributing to chromosomal instability. However, it seems not to affect hematopoietic neoplasia, which suggests that other factors are important in chromosomal instability and induction of translocations.

A large number of genes have been studied for hypermethylation and silencing in hematopoietic neoplasia, mostly in adult patient groups. Focus has been on genes of cell cycle regulation, apoptotic pathways, genes involved in growth and differentiation, since it seemed plausible that disruption of these genes could confer neoplastic alterations. The most reported silencing is for the cyclin-dependent kinase inhibitors p15^{INK4B} and to a lesser extent for p16^{INK4A}, and both genes have been found silenced in ALL and AML. In adults, the majority of AML and ALL have loss of p15 and 20-40 % loss of p16. In pediatric patients, the number of studied patients is far less, but it seems that p15 is methylated, mainly in AML in less than 50%, frequently in T-ALL, but hypermethylation of p16 does notseem important. Other genes reported hypermethylated and silenced at least 30% of samples in mostly adult studies include: Calcitonin, E-cadherin, Estrogen Receptor, MyoD, HIC1, MDR1, plTX2, SDC4, WT1 ([9-11]). In adult studies, an unfavorable prognostic association has been observed for patients with > 2 hypermethylated genes. For pediatric patients this has not been confirmed, possibly because most studies contained few patients and also hypermethylation did not seem to be as frequent in pediatric AML/ALL, at least for the genes studied [12]. Genome wide methylation studies have not been performed in pediatric AML/ALL.

MLL rearranged infant ALL seems characterized by hypermethylation and silencing of the FHIT gene. FHIT had already been found genetically altered and silenced in several human malignancies. Silencing by hypermethylation was observed in 40% of ALL cell lines and 27% of pediatric ALL samples [13], especially in hyperdiploid tumors. Stam et al[14] found it to be specific for MLL rearranged infant ALL, since 100% of MLL+ samples were hypermethylated and silenced for FHIT versus 60% in a mixed group of MLL- infant ALL and non-infant ALL. The FHIT expression was inducible after treatment

with the demethylating agent decitabine and over expression of FHIT induced apoptosis and leukemic cell death. These data suggest that FHIT acts as a tumor suppressor gene, and may be characteristic for certain ALL subgroups such as MLL+ infant ALL.

Acute promyelocytic leukemia (APL) has long been the role model of epigenetic silencing in leukemias, although the mechanism of silencing is different. The disease is characterized by the inactivation of the retinoic acid receptor-beta (RARâ) by a HDAC repressive complex. The APL specific fusion protein PML/RARá has the ability to suppress RARâ by recruitment of a nuclear co-repressor -histone deacetylase (NCOR/HD) complex and DNMT3a, resulting in local chromatin remodeling. Treatment of cells with all trans retinoic acid (ATRA) induces demethylation of the promoter, with gene re-expression and reversal of the malignant phenotype by differentiation. In a transgenic mouse model and in clinical studies with refractory APL patients, silencing of RARâ can also be reversed by the HDAC inhibitor sodium phenyl butyrate (SPB)[15] .

Epigenetic drugs

DNA methylation and chromatin remodeling are involved in important mechanisms, which are believed to contribute to cancer. Contrary to genetic alterations in cancer, DNA methylation and histone deacetylation are reversible with restoration of the normal gene transcription. This is especially applicable for transcription restoration of silenced tumor suppressor genes by regional hypermethylation. Two classes of drugs will be discussed: Demethylating agents and Histone De-Acetylase Inhibitors (HDACi):

Demethylating agents

Clinical testing of two azanucleosides already started in the 1970s. They were tested as novel cytosine analogues and at comparatively high doses seemed to have the same effect and toxicity as cytarabine/ara-C and since cytarabine was already widely used the testing of the azanucleosides was interrupted (reviewed in [16]). However, 5-aza-cytidine (Vidaza) and the more potent 5-aza-2'-deoxycytidine (Decitabine) were revived as DNA demethylating agents (figure 2). Reactivation of silenced genes by using these compounds was shown *in vitro* for many different situations, for instance in the hypermethylated CGG expanded repeats in the fragile X syndrome, in the silenced copy of the SNRPN and neighboring genes in cell lines of Prader-Willi patients, but also in many aberrantly hypermethylated genes in different cancer types. Clinically significant results have been obtained in myelodysplastic syndrome (MDS), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML)[17, 18]. In MDS, demethylation of the tumor suppressor gene p15 was observed in several patients and correlated with clinical response. Vice versa, the increase in methylation of p15 in one patient correlated with disease progression. Interestingly, dose de-escalating testing showed that Decitabine was most effective in the lower or middle doses, with low or minimal non-hematologic toxicity.

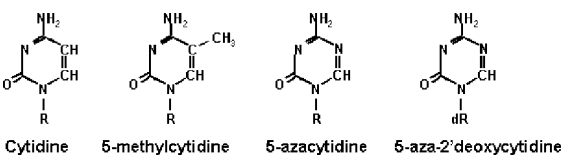

Figure 2: *Chemical structure of cytosine and analogues. R, Ribose; dR, deoxyribose*

HDACi

Deacetylation, especially of the H3K9 is essential in the formation of heterochromatin and silencing of genes. The Histone Deacetylase enzymes (HDACs) are the driving force of deacetylation processes and they can pharmacologically be inhibited. Currently, several structurally diverse classes of HDACIs, natural and synthetic, are known to bind histone deacetylases (HDACs) and to induce histone acetylation. These include short-chain fatty acids (i.e. valproic acid, sodium butyrate, phenylbutyrate and AN-9), cyclic and non-cyclic hydroxamates (i.e. suberoyl anilide hydroxamic acid (SAHA), trichostatin A (TSA), *m*-Carboxycinnamic acid bishydroxamic acid (CBHA), suberic bishydroxamic acid (SBHA), oxamflatin, proxamide, NVP-LAQ824 (LAQ824), and PDX101), cyclic peptides or tetrapeptides (i.e. Depsipeptide (FK228), Trapoxin, and

apicidin), benzamides (i.e. MS-275 and CI-994 (*p-N*-acetyl dinaline)), ketones (i.e. trifluoromethyl ketone and á-ketoamides), and hybrids of hydroxamic acid and cyclic tetrapeptide (i.e. CHAP). Clinical testing of HDAC inhibitors was first applied effectively in acute promyelocytic leukemia (APL), in which RARâ is inactivated by an HDAC repressive complex[15]. Currently, a wide range of different HDAC inhibitors alone or in combination with demethylating agents are in pre-clinical and clinical phase I/II trials. The toxicity seems very limited, without unexpected toxicity reported, but the effectiveness has not been completely established yet.[19, 20]. In vitro, there is extensive evidence that HDACi induce cancer cell cycle arrest, growth inhibition, differentiation, and programmed cell death[21, 22].

Combining demethylating agents and HDACi

In a landmark paper by Cameron *et al* a synergistic effect of histone deacetylase inhibition and demethylation was shown[23]. TSA and decitabine were both able to reverse gene silencing of several hypermethylated genes in colon cancer cell lines. However, the combination of the two agents showed a much more significant demethylation and reactivation of the genes. This synergistic effect of a demethylating agent and an HDACi was confirmed in further studies[24, 25]. In leukemias, a phase I/II study has shown that using decitabine and valproic acid in adult patients induced responses and remissions in pre-treated and chemotherapy-naïve patients[26].

Conclusions

The level of transcription of genes can be altered by genetic alterations, like mutations, translocations or aneuploidy. However, epigenetic alterations play an important role in human malignancies as well. Especially regional hypermethylation and silencing of genes have become a hallmark of cancer. Also in (pediatric) leukemias, multiple presumed tumor suppressor genes have been found silenced by promoter hypermethylation. The hypermethylation is part of complex chromatin remodeling mechanisms. It has become evident that demethylating agents, HDAC inhibitors or a combination of both, can reverse both methylation and the formation of repressive chromatin. The future will teach us the value of these epigenetic drugs, since many clinical phase I/II studies are on the way in adult patients with a variety of cancer types. These data will create opportunities to design similar epigenetic drug studies in pediatric patients.

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Treatment of MLL gene rearranged Acute Lymphoblastic Leukemia

Rob Pieters

Introduction

Infant leukemia is defined as leukemia occurring in the first year of life. Whereas ALL has a higher incidence than ANLL in older children, the incidence of ALL and ANLL in infants is about equal. Infants account 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]. 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 ANLL and never ALL.

At least two independent mutations are necessary to cause leukemia. The first is thought to take place in utero for the majority of childhood leukemia cases in general whereafter postnatal events are required for the full development of ALL. However, in infant leukemia, all necessary genetic events may have occurred in utero. Indirect evidence for this hypothesis includes the sometimes very early onset of infant leukemia and the high rate of concordance of leukemia in identical monozygotic twins if one of the children developed leukemia in the first year of life [2,3] Direct evidence for the prenatal origin of infant ALL was demonstrated by Gale et al [4] who detected unique *MLL-AF4* fusion sequences in the Guthrie cards from infants who developed ALL at very young age.

Clinical Presentation and Biology

Compared to older children with ALL, infants have a high leukocyte count and an increased incidence of hepatosplenomegaly and central nervous system (CNS) involvement . In about half of the infants with ALL, the WBC is >100 x 10^9/L. About 15% of infant ALL cases have CNS involvement at diagnosis. Enlarged testes were seen in ~13% of male infants. The mediastinum is enlarged in less than 5% of infant ALL cases. [1]

Immunophenotype

About two-thirds of infant ALL is classified as very immature CD10- negative B-lineage precursor ALL (proB ALL). The remaining group mainly consists of common/pre-B cases. Mature B-lineage ALL is an exceptional finding, while T-lineage ALL was diagnosed in only 4% of cases [1]. Infant ALL cells are more likely to express myeloid-associated antigens. These data suggest that infant ALL arises from an immature precursor cell that is not fully committed to lymphoid differentiation. Intraclonal switch from B-lineage to monocytic lineage leukemia has been described in infants. [5]

Genetics

Cytogenetic abnormalities that occur relatively frequent in older children, such as hyperdiploidy and the t(12;21) resulting in the *TEL/AML1* fusion product, but also the Philadelphia translocation t(9;22) and the t(1;19), are rare in infant ALL. The most common chromosomal aberrations in infant ALL are translocations involving chromosome band 11q23 leading to translocation of the MLL gene. Cytogenetic analysis detects MLL gene rearrangements in ~50% of infant ALL cases, but this percentage rises to 80% when molecular techniques are used. The t(4;11)(q21;q23) is the most common and is found in about 50% of the MLL gene rearranged cases whereas the t(11;19)(q23;p13) in about 20% and the t(9;11)(p22;q23) in 10%. More than 40 other 11q23 partner chromosomes have been reported, all occurring at a very low frequency but together in about 10-20%. Therefore, most *MLL* gene rearranged cases will be detected if specific RT-PCR's for the well-known t(4;11), t(9;11) and t(11;19) fusion

transcripts are performed but other translocations will be missed. The split-signal FISH method is an easy method to detect any types of MLL gene translocation in a single FISH experiment and is therefore advised as a first screening technique [6]. It requires a low amount of material, but does not show which MLL gene rearrangement is present.

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.. About 20% of infants with ALL lack these features. The poor prognosis of infant ALL has been associated with the following factors in univariate analysis ([1,7]: age below 3 or 6 months, organomegaly, CNS involvement, a high WBC, CD10 negativity, myeloid antigen expression, MLL gene rearrangement and day-14 bone marrow or day 8 response peripheral blood response to therapy. Gender was not a prognostic factor. In multivariate analysis including these factors, the presence of MLL rearrangements often remains the most important factor predicting a poor outcome [8, 9, 10]. The EFS for MLL rearranged infant cases (5–34%) is worse than for their MLL germ line counterparts (42–92%) [7]. Some studies reported that only t(4;11) positive infant ALL patients experience a poor prognosis, whereas patients carrying other types of MLL rearrangements fare equally well as MLL germ line cases [10, 11]. In a large meta-analysis, all types of MLL gene rearrangements were associated with a poor prognosis in infants [12]. In a recent review [7] we concluded that the presence of MLL rearrangements and young age are probably the strongest independent prognostic factors. This is confirmed by preliminary results from the large collaborative Interfant-99 study.

Biology and drug resistance

Leukemic cells from infants with MLL gene rearranged ALL cells grew better on stromal cell layers in vitro [13], had a higher leukemic cell recovery when inoculated into SCID mice [14] and were more resistant to cell death resulting from serum deprivation in vitro [15] compared with cells from other children with ALL. Infant ALL cells were more resistant in vitro to prednisolone and L-asparaginase than cells from older children with ALL [16]. This is in concordance with the finding that infants with ALL more frequently show a poor response to prednisone than older children with ALL do [17, 18]. Infant ALL cells do not express higher levels of the multidrug resistance genes BCRP, MDR1, MRP1 and LRP/MVP than other ALL subtypes. [19]

Although relatively resistant to several chemotherapeutic drugs, infant ALL cells are more sensitive to the cytarabine (Ara-C) and the adenosine analogue 2-CdA (2-chlorodeoxyadenosine or cladribine) compared with cells from older children with ALL [16. 20]. 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* [21]. The Ara-C sensitivity is most likely due to the high expression of the human equilibrative nucleoside transporter 1 (hENT1) [22], 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 [23, 24]. Also, improved outcome for adult pro-B ALL cases was observed with intensified post-remission therapy including high-dose Ara-C/mitoxantrone [25]. In 1999, the collaborative Interfant-99 treatment protocol for infant ALL was initiated that added the intensive use of both low and high-dose Ara-C on top of a ALL based chemotherapy schedule. These observations may also the use of the combination of Ara-C and 2-CdA in infant ALL. Moreover, given the sensitivity of infant ALL cells to nucleoside analogues, newly developed nucleoside analogues, e.g. clofarabine and troxacitabine, may be interesting candidate drugs for further analysis in infant ALL.

Treatment results

Whereas the overall cure rate for childhood ALL has risen to ~80% with contemporary treatment schedules, progress in the treatment of infant ALL has remained behind. We recently reviewed the results of published studies, showing an

overall EFS rate of 35-40%. [1, 7] The complete remission rate in infant ALL is about 94%. Toxicity after remission induction is not the major problem: ~4% of infants die from therapy toxicity. 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. Outcome after these early relapses is very dismal. So early bone marow relapse is the major cause of death in infant ALL

Comparison of treatment protocols

Comparisons of different treatment protocols and outcome are difficult because most protocols differ in many details and the numbers of included patients are low, even in the larger published trials.

A study performed by several POG institutions, resulted in a 5-year EFS of only 17%. [26] Unlike other protocols, this regimen did not contain dexamethasone, high-dose methotrexate (MTX), high-dose cytarabine (ara-c), cyclophosphamide or ifosfamide and L-asparaginase was used in the induction phase only. In another POG study (8493), (27) 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 in contrast to other protocols.

Protocols of MRC UKALL specified high-dose MTX dose and high-dose ara-c, but not dexamethasone, cyclophosphamide or ifosfamide. [28] L-asparaginase was administered only in the induction phase. The overall EFS rate was 25%, lower than that reported by other groups. The Dana-Farber Cancer Institute (DFCI) consortium has intensified its treatment protocols since 1985, leading to a significant improvement in treatment results.[23] The main difference with the historical control series was the introduction of a postinduction intensification phase including high-dose MTX, high-dose ara-c, L-asparaginase, vincristine and 6-mercaptopurine. Dexamethasone, cyclophosphamide / ifosfamide, and epipodophyllotoxins were all excluded from DFCI protocol since 1985. Cranial irradiation was administered at the age of 1 year. These data should be looked at with caution because of the limited number of infants enrolled.

Since 1983, BFM investigators stratified patients according to the prednisone response and presenting leukemic cell burden, resulting in diverse treatments for infant. Infants were over-represented in the higher-risk arms because of their leukemic burden and high number of patients with a poor prednisone response. The overall EFS rate for infants, 43%, is among the highest of reported results [18]. A small study of the EORTC–CLG [29] using a slightly modifed BFM regimen also resulted in a 43% EFS. In both studies, cranial irradiation was given to a subgroup of the the patients.

The CCG-1883 resulted in 39% EFS, which is comparable to the results reported by BFM and DFCI investigators. [24] These outcomes were better than historical CCG control series in which less intensive systemic therapy was used. Importantly, the CNS relapse rates in the more recent studies, which relied on intensive systemic chemotherapy and intrathecal therapy, but without irradiation, were no higher than those in trials with radiation. Major difference between the CCG protocols described by Reaman [24] compared to historical controls was the inclusion of high-dose ara-c, cyclophosphamide, and more L-asparaginase in the consolidation and reconsolidation phases

An important finding was that cranial irradiation and intensive chemotherapy combined with intrathecal therapy result in the same CNS relapse rate, even in patients with CNS involvement at initial diagnosis. [24] In particular, high-dose MTX, high-dose ara-c, dexamethasone, and intrathecal therapy may contribute to prevention of CNS relapses.

Another possible conclusion from a comparison of historical controls, intensive POG protocols, and more recent protocols of the CCG, BFM and DFCI is that intensive postinduction chemotherapy and the use of high-dose ara-c, high-dose MTX, L-asparaginase, dexamethasone and cyclophosphamide/ ifosfamide are helpful in preventing early bone marrow relapses. In the context of the low incidence of infant ALL, large international collaborative studies are needed to study new

therapies for this disease. Two large collaborative efforts - COG and Interfant - are currently analyzing the efficacy of intensified therapy for infant ALL.

Bone marrow transplantation

The role of allogeneic bone marrow transplantation (BMT) in infant ALL is unclear and debatable. No proper randomized studies have been performed comparing allogeneic BMT with chemotherapy, so the published data are scant and selective. A relatively large meta-analysis [12] did not show a benefit for the use of allogenic BMT from a matched donor in infant MLL gene rearranged ALL. Controlled studies are needed to determine the usefulness of BMT in this vulnerable group of patients. In the Interfant studies BMT is restricted to infants with a very high risk of relapse.

Late effects

Little is known about late effects of treatment for infant ALL, mainly because substantial numbers of infants did not survive until recently. In studies reported to date, learning disabilities and developmental delays were identified in the majority of irradiated infants. [23, 29] Obesity and short stature were found in ~25% irradiated cases. Asymptomatic echocardiographic abnormalities and stable congestive heart failure have been reported in single cases. [23, 29] In 30 nonirradiated infants who were treated with high-dose MTX as CNS-directed therapy, the neurodevelopmental outcome was normal. [30] Frankel [27] reported on one patient with a severe developmental disorder among 18 infants who were neither irradiated nor transplanted and remained in complete remission. As treatment becomes more effective for infants with leukemia, it will be important to incorporate prospective studies of late effects into all new protocols.

Drug Dosage Adjustment

A persistent problem are the rules for drug dosage adjustment in infants. [31] The total-body water content decreases from 75% at birth to 60% at 1 year, and the percentage of extracellular water decreases with age. [32] Drugs bind less avidly to serum proteins in newborns than in adults, leading to a higher unbound active fraction of drugs in infants. [33] The lower activity of P-450 enzymes in infants [34,35] 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 [31]. 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. [36] 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.

A small study in infants with ALL showed no decreased clearance of MTX compared to older children. [37] 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. [38] Others have not found a difference in ara-C clearance between infants and older children. [39] At the moment, current protocols rely on arbitrary calculations based on body weight, body surface area or one of these with a correction for age. Thus, pharmacokinetic studies together with toxicity measurements are urgently needed in infants with leukemia (and other types of cancer).

New therapeutic targets

Combinations of multiple new drugs will be required to cure infant MLL gene rearranged ALL patients who are not cured with current chemotherapies. Thus, several innovative treatment strategies are needed that either overcome resistance to conventional drugs or which involve alternative novel agents that more effectively target infant MLL cells. [7] Examples of these are mentioned below.

In addition to ara-C and 2CdA described above, a class of nucleoside analogue drugs that may be effective against MLL gene rearranged ALL cells are DNA demethylating cytidine analogues, such as 5-azacytidine, 5-aza-2'-deoxycytidine (decitabine), or the recently identified agent zebularine [40, 41] showed that *MLL* rearranged ALL cases had the highest number of methylated genes of all ALL subtypes. Thus, MLL gene rearranged ALL is characterised by aberrant DNA

hypermethylation. In concordance with this, we recently 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 observedin obnly 50% of older children with ALL [42]. We observed that 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. Therefore, inhibition of DNA methylation may be an effective therapeutic strategy in the treatment of infant MLL, especially since decitabine depends on ENT1 to cross the cell membrane, which is highly expressed in infant ALL cells [22].

FLT3, the gene encoding Fms-like tyrosine kinase 3, is highly expressed in patients with MLL gene rearranged ALL [43]. FLT3 is important in early B-lineage development and is highly expressed in immature B-cells [44]. In AML the *FLT3* gene is frequently subjected to mutations that activate this receptor [45]. 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 leukaemic cell death. This has led to the initiation of phase I/II clinical trials with these inhibitors in refractory adult AML, and so far the results are promising [46, 47, 48]. 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* [21, 49]. 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 [21, 50]. This showed that FLT3 inhibition may represent a novel therapeutic strategy for infant MLL that needs clinical testing.

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 of infant ALL is the occurrence of early relapses, justifying early intensive chemotherapy. The role of allogenic bone marrow transplantation in infants is debatable. Large collaborative studies are the only way to investigate possible improvements of therapy for infants with ALL. Development of new innovative approaches are needed to increase the cure rate to the same rate as that in older children with ALL.

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Treatment of Philadelphia positive (Ph+)ALL and CML in children

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Introduction

Philadelphia positive (Ph+) leukemias in children include essentially all chronic myeloid leukemias (CML) and 2% to 3% of childhood acute lymphoblastic leukemias (ALLs). Ph+ leukemias are characterized by a reciprocal translocation between chromosomes 9 and 22 (Philadelphia chromosome). The translocation creates a fusion of human homologue of the Abelson Murine leukemia virus *ABL* on chromosome 9q34, with breakpoint cluster region *BCR* on 22q11 (reviewed in ref. 1) An in frame *BCR-ABL* fusion transcript results in the upregulation of the abl tyrosine kinase. Primarily, three main *BCR-ABL* chimeric transcripts arise from distinct breakpoints in the *BCR* gene, resulting in fusion of the *BCR* exon 1, exon 1-12/13 or exons 1-19 to *ABL*. The molecular masses of the protein products are 185/190, 210 and 230 kDa respectively. In most patients with CML and in approximately 10% of patients of ALL it is the $p210^{BCR-ABL}$ product which is produced, although low levels of the $p185^{BCR-ABL}$ product are often detect in CML patients. For the majority of Ph+ALL and in occasional patients with CML, a $p185^{BCR-ABL}$ product is found. The alternative translocation product, *ABL-BCR* can be detected but is not thought to play a role in leukemogenesis [1].

The bcr-abl fusion proteins are characterized by a constitutive protein tyrosine kinase (PTK) activity that is absent in the normal abl protein. This dysregulated PTK activity, which results in changes of multiple signal transduction pathways, is crucial to the transforming activity of the bcr-abl fusion proteins and their ability to cause leukemias in vivo [1]. Therefore, inhibition of the PTK activity of this oncoprotein is a rational therapeutic approach for *BCR-ABL* expressing leukemia.

Imatinib is an inhibitor of the protein-tyrosine kinases associated with bcr-abl, the platelet-derived growth factor (PDGF) receptor and c-Kit, but not of other members of the Type III receptor kinase family, such as Flt-3 and Fms [2,3]. Imatinib shows selectivity for the Abl protein-tyrosine kinase at the *in vitro*, cellular and *in vivo* levels. The compound specifically inhibits proliferation of bcr-abl expressing cells. In colony forming assays using *ex vivo* peripheral blood and bone marrow samples, Imatinib shows selective inhibition of bcr-abl positive colonies from CML patients. In animal models, the compound shows potent anti-tumor activity against bcr-abl and v-abl expressing cells at tolerated doses. Imatinib has dramatically changed the treatment of adult Ph+ CML [1-3] and showed promising results in adult Ph+ALL in combination with chemotherapy [4].

Ph+Acute lymphoblastic leukaemia (ALL)

Recent advances in treatment have increased the cure of childhood ALL to 75 percent or better [5,6]. However attempts to improve results for resistant subtypes of ALL, such as Ph+ ALL, have been largely unsuccessful. Overall Ph+ALL, which accounts only for 2-3% of children with ALL have a dire prognosis (rates of EFS are 25-30% in children and even less in adults)[7]. Some investigators suggest that Ph+ALL in childhood is heterogeneous with regard to sensitivity to treatment. Good initial response to steroids (which are given in combination with intrathecal methotrexate before induction chemotherapy is instituted) as well as age and leukocyte count at diagnosis, have been shown to correlate with a good clinical outcome in children treated only with chemotherapy [7-9]. The heterogeneity of Ph+ALL with respect to clinical outcome has been recently confirmed by the analysis of the largest series of pediatric ALL treated by 10 European and United States study groups or large single institutions from 1986 to

1996 [7]. Among patients who presented with WBC higher than 100 x 10^9/l, 85% did not have long-term EFS at five years. The inadequacy of current therapy for such patients, most of whom can be readily identified by their initial response to prednisone, indicates a need for new treatments. Patients who are younger than 10 years old and have a WBC less than 50 x 10^9/l at the time of diagnosis have about a 50 percent chance of long term DFS whereas the remaining patients (those with WBC of 50 x 10^9/l to 100 x 10^9/l and those with less than 50 x 10^9/l leukocytes who are older than 10 years of age) have an intermediate prognosis (estimate of five-year DFS, 30 %) [7].

The prognostic impact of early response to induction was recently reported by the UK Medical Research Council ALL trial for childhood ALL,MRC ALL97 [10]. Between January 1997 and June 2002. Forty-two (2-3%) patients were Ph+. Nineteen (45%) had <25% blasts in bone marrow (BM) within the first 2 weeks of treatment and were defined as a good response group (GRG), the others as a poor response group (PRG). Thirty-six (86%) achieved first complete remission (CR1) at the end of induction, of which 28 underwent BM transplantation (BMT). The median follow-up was 42 months (range, 21–84). The 3-year event-free survival (EFS; 52%, 95% CI, 36–66%) was a considerable improvement on the previous MRC UKALL XI trial (27%). EFS for the GRG and PRG were 68% (43–84%) and 39% (18–59%), respectively (P=0.03); presenting white cell count <50x10^9/L (P=0.02) was predictive for overall survival.

Stem cell transplantation from HLA-matched related donor yields a significant better outcome than chemotherapy alone [7,11]. The absence of any significant superiority to chemotherapy in patients undergoing SCT from a mismatched donor or matched unrelated donor (MUD), could be explained by the high number of transplantation-related deaths, reported in this study [11]. In most recent years better results have been obtained with unrelated donor HSCT, in series which include either children and adults [12]. The leukemic cell burden present before HSCT influences the rate of relapse-free survival: patients with detectable BCR-ABL-expression prior to HSCT have a significantly worse prognosis.

Scanty are the information on efficacy of Imatinib in pediatric Ph+ ALL, but according to the data obtained so far in adult patients with acute Ph+ leukemia or blast crisis of CML, the drug produce only a transient effect when used alone (4 and reviewed in ref.13). Accordingly, most ongoing trials in acute Ph+ leukemia, are exploring the possibility to incorporate Imatinib in the context of multi-agent approach (either in combination or during the interval of chemotherapy blocks). One group used a hyper-CVAD (fractionated cyclophosphamide, vincristine, Adriamycin and dexamethasone)-type regimen[14] and incorporated imatinib postinduction. They achieved a CR rate of 80%[15]. Although approximately 50% of their refractory patients ultimately achieved CR when Imatinib was used postinduction, this is not significantly different from previous results without Imatinib. Two groups have used a similar hyper-CVAD regimen, but with the concurrent use of Imatinib during remission induction [16,17]. All patients in these two studies were in CR at the end of induction and a high rate of molecular CR was achieved prior to allo-SCT. All three groups reported a high success rate post-allo-SCT when compared with historical controls and, reassuringly, the use of Imatinib does not appear to increase transplant-related morbidity.

Two cooperative studies are currently ongoing to test the relevance of Imatinib incorporation on intensive chemotherapy backbone for treatment of childhood Ph+ALL. In the AALL0031, from the Children's Oncology Group (COG), the protocol chemotherapy is based on various promising components previously studied in other ALL COG trials. This includes the use of ifosfamide and etoposide from POG ALL relapse studies, the successful use of high dose methotrexate for ALL, and the CCG NY II regimen used in high risk ALL. Imatinib was planned to be gradually introduce in cohorts of patients in this study, with later cohorts receiving it in more phases of therapy if earlier cohorts are treated successfully without toxicity problems. Also, the subset of patients in this study who have an appropriate donor was eligible to receive hematopoietic stem cell transplant (HSCT) following the initial two courses of consolidation therapy in the study. The study was opened to patient entry on October 14, 2002 and based on

most recent accrual data, the study should reach its Ph+ target accrual goal by approximately May 2006 (Kirk Schultz, personal communication, January, 2006). All patients in the current European collaborative trial for Ph+ ALL in childhood (EsPhALL) are receiving remission–induction therapy as per national study group recommendations for children with ALL. Postinduction, all treatment will be on a common European collaborative protocol. All good responders are randomised to receive or not receive imatinib in addition to standard chemotherapy. For those receiving a prednisolone prophase, a good-responder is defined as one with a peripheral blast count of $<1x10^{9}$/l at the end of a week. For children on other protocols, this is defined as blast count of <25% in a bone marrow aspiration taken during the first 2 weeks of induction. All other children are defined as poor-responders and receive Imatinib along with standard chemotherapy. The study was opened in 2004 and it will be completed in 2007.

Chronic myelogeneous leukaemia

Diagnosis of CML is rare in childhood; it accounts for only 3 % of leukemias below the age of 18 yrs. Thus, data describing diagnostic features and the natural history of the disease are mostly depicted from small series of cases [18,19]. In children the course of the disease seems not to differ from adult patients. However, the younger age generally qualifies pediatric patients as candidates for hematopoietic stem cell transplantation.Although treatments such as hydroxyurea and interferon alpha (IFNa), with or without cytarabine, may induce responses, they are not curative [20].

Similar to adult CML, the only potentially curative therapy for childhood Ph+ leukemias remains allogeneic stem cell transplantation, which is most effective with a matched sibling donor during the chronic phase of CML [21-25]. Unfortunately, high rates of transplant-related mortality (approximately 20%) and posttransplantation recurrence (17%) may occur [21].

The substantial antileukemic activity of Imatinib in adult CML trials prompted investigators in the COG to evaluate this agent in children with recurrent or refractory Ph+ leukemias. The objectives of this phase 1 study were to determine the optimal dose of Imatinib for phase 2/3 pediatric trials, to evaluate the toxicities and plasma pharmacokinetics of Imatinib in children, and to provide a preliminary evaluation of the antileukemic activity of Imatinib in Ph+ childhood leukemias [26]. The purpose of the study was to determine dose-limiting toxicities in children with refractory or recurrent Ph+ leukemias. Oral Imatinib was administered daily at dose levels ranging from 260 to 570 mg/m^2. There were 31 children who received 479 courses of Imatinib. The most common toxicities encountered, which occurred in less than 5% of courses, were grade 1 or 2 nausea, vomiting, fatigue, diarrhea, and reversible increases in serum transaminases. One patient at the 440-mg/m^2 dose level had dose-limiting weight gain. There were no other first-course dose-limiting toxicities. A maximum tolerated dosage was not defined. Among 12 CML patients evaluable for cytogenetic response, 10 had a complete response and 1 had a partial response. The Authors found that daily oral Imatinib is well tolerated in children at doses ranging from 260 to 570 mg/m2. Doses of 260 and 340 mg/m2 provide systemic exposures similar to those of adults who are treated with daily doses of 400 and 600 mg, respectively.

Although hematopoietic stem cell transplantation remains the treatment of choice for children with Ph leukemias whose physiologic state permits intensive therapy and for whom donors are available, Imatinib mesylate is an important option for facilitating induction of complete remission in children with recurrent or refractory disease or for whom there is not a suitable donor. On the basis of the results of our study and the favorable results obtained with newly diagnosed adult patients with CML [27], several cooperative pediatric study groups are currently evaluating the role of Imatinib in children with newly diagnosed Ph+ CML.

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New molecular targets for therapy in leukaemia

Pamela Kearns

Increasing the understanding of the molecular basis of leukaemogenesis and the cellular processes which maintain the malignant phenotype has led to the hope that we will be able to harness this knowledge in the development of exquisitely targeted therapies. Defining a theoretically ideal target for therapeutic exploitation is relatively straightforward but realising this ideal remains a considerable challenge. An ideal therapeutic target would be clone-specific, intrinsic to the proliferative or survival capacity of the cell, readily targeted by molecules that are easy to synthesise, bioavailable and would not develop a resistance mechanism.

The specific challenge for targeted drug development in leukaemia is the inherent heterogeneity of the disease. Acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) comprise a wide range of phenotypic and genotypic sub-types. Moreover, within a single patient, sub-populations of leukaemia are identifiable, both immunophenotypically and cytogenetically. There is increasing evidence of sub-populations of leukaemic cells with properties of self-renewal, multi-potentiality and proliferative capacity, which are believed to constitute the so-called 'leukaemia stem cells'. To date, this has been best characterised for AML and chronic myeloid leukaemia (CML). The leukaemia stem cells exhibit different immunophenotypes from the bulk population of leukaemia cells and unique molecular features are being defined. For example, constitutively high levels of NF-•B activity have been reported in the proposed AML stem cell. High levels of NF-•B activity could convey a survival advantage to the stem cell and may be responsible for persistence of the stem cell and subsequent relapsed disease and therefore could be an attractive target for therapeutic exploitation. Defining molecular characteristics specific to the leukaemia stem cell may provide important novel approaches to treatment in the future.

The most successful and advanced example of a targeted approach to the treatment of leukaemia is provided by the BCR-ABL kinase inhibitor imatinib (STI-571, Gleevec™). The fusion protein BCR-ABL comes close to fulfilling the requirements of the ideal target and imatinib is often held up as a paradigm of targeted drug development. The chimeric activated kinase produced by the translocation of the ABL gene on chromosome 9 to the BCR gene on chromosome 22 is exclusive to leukaemia cells and is pivotal to cell survival in CML. The active site on the BRC-ABL protein is readily inhibited with a low molecular weight, water soluble drug, however, the development of imatinib resistance due to kinase domain mutations is now well recognised. Nevertheless, this model approach has led to the rapid expansion of small molecules targeted at receptor tyrosine kinases, some of which are proving of therapeutic interest in childhood leukaemia. Dual kinase inhibitors, for example the SRC/ABL kinase inhibitor, dasatinib have been shown to be highly effective in imatinib resistant leukaemias, with potency against the majority of BCR-ABL kinase domain mutations, the notable exception being the T3151 mutation, seen in 10-15% of imatinib resistant CML.

The increasing number of studies investigating the genotypic heterogeneity in leukaemia have translated into the identification of a high number of potential molecular targets, many of which do not have the level of disease specificity associated with the receptor tyrosine kinase activity of BCR-ABL, nonetheless, they are proving important therapeutic targets. Common themes in the function of these potential therapeutic targets are emerging, including signal transduction pathways implicated in the pathogenesis of the disease and activated

pathways conveying proliferative or survival advantage to the leukaemia cell.

The concept of targeting transcription regulatory proteins is emerging from greater understanding of the co-ordinated events surrounding transcriptional activation and repression. In common with the other potential therapeutic targets described, aberrant transcriptional regulation in haematological malignancies can enhance cellular proliferation and convey a survival advantage to leukaemic cells. Recognised oncogenic transcription factors involved in haematological malignancies, include AML-1/ETO, CBF•/MYH11 and PML/RAR• The use of the retinoic acid ATRA in acute promyelocytic leukaemia (APML) which is characterised by the chimeric transcription factor PML/RAR••• well a described model for transcriptional targeting. An alternative approach to transcriptional targeting is to target proteins involved in chromatin re-modelling, namely DNA and histone methyltransferases and histone deactylases, although this approach is inherently less disease specific.

Aberrant activation of signal transduction pathways, which may arise from gene mutations or deletions are proving a rich source of potential therapeutic targets in haematological malignancies. Mutated components of activated pathways may be targeted directly or indirectly via downstream components of the pathway. This approach is exemplified by the activating mutations described in the FMS-like receptor tyrosine kinase 3 (FLT3). FLT3 is normally expressed in normal bone marrow, in the CD34+ haemopoietic progenitors. It is one of a family of class III receptor tyrosine kinases of which other family members include PDGFR, KIT and FMS. Ligand binding to the FLT3 receptor induces dimerisation and autophosphorylation leading to phosphorylation of a cascade of downstream signalling pathways, which involve RAS-ERK, PI3K-Akt and signal transducer and activator of transcription 5 (STAT5), all of which are involved in cellular proliferation, differentiation and survival. Aberrantly expressed FLT3 activity can arise from over-expression of the wild-type FLT3 and its ligand, or from two types of activating mutations; internal tandem repeats (ITD) in the juxtamembrane domain and point mutations in the so-called 'activation loop' of the kinase domain. Both types of mutation result in aberrant activation of the tyrosine kinase activity and consequent activation of intracellular signalling pathways leading to cellular proliferation and survival advantage. Activating mutations are found in both adult and paediatric leukaemias, predominantly in AML. In childhood AML activating mutations are reported in 18-25% of cases, 10-17% due to ITD. FLT3 over-expression is described in childhood pre-B ALL and infant ALL when associated with gene rearrangement involving the *MLL* gene at 11q23 and in 16% of cases, activating point mutations are described. Overall, aberrant FLT3 activation is associated with poor prognosis and therefore defines an important patient group for improved therapies. Several small molecule FLT3 kinase inhibitors have been developed and are in different stages of clinical development, including the staurosporine derivative PKC412 and the inodolocabazole derivative CEP-701. Like imatinib they act via competitive binding to the ATP binding site. FLT3 as the crucial target for these small molecule inhibitors is supported by evidence from pre-clinical studies. Both CEP-701 and PKC412 selectively inhibited the proliferation of the murine lymphocyte cell line Ba/F3 when transfected with FLT3 carrying the ITD mutation and were shown to inhibit FLT3 autophosphorylation and in vitro tyrosine kinase activity. *In vivo* studies have demonstrated that bone marrow from Balb/c mice transduced with a FLT ITD containing retrovirus caused a lethal myeloproliferative disorder when transplanted into healthy irradiated mice. CEP701 and PKC412 were shown to prolong survival of mice with FLT ITD transduced bone marrow. Notably, neither of the two inhibitors described above exclusively inhibit FLT3, for example PKC412 also effectively inhibits VEGFR, PDGFR and KIT and CEP701 inhibits Trk-A and to a lesser extent PDGFR• and FMS. The clinical importance of this lack of specificity remains to be evaluated. Clinical development to date has been in adult leukaemias, principally AML and early data suggest promising activity with tolerable toxicity profiles. The initial data suggest that FLT3 inhibitors are unlikely to produce sustainable responses as single agents and their development should focus on combination therapies. Clearly, development of these agents in selected paediatric patients would be of interest.

Other signal transduction pathways, implicated in cellular proliferation and survival include the RAS mediated and the PI3K-Akt / mTOR pathways. Several studies have identified components of both these pathways as potential therapeutic targets in the treatment of leukaemia. Both the BCR/ABL inhibitor imatinib and the FLT3 Inhibitors target kinase activity, therby inhibiting protein phosphorylation. Other post-translational modifications to proteins are increasingly recognised as essential to their function. The role of protein prenylation is emerging as an important post-translational modification with relevance to protein within signal transduction pathways. Prenylation is a lipid modification involving the cholesterol biosynthesis intermediates, 15-carbon farnesyl and 20 carbon geranylgeranyl, catalysed by 3 prenyltransferases; farnesyltrasnferase and geranylgeranyltransferases I and II. Important farnesylated proteins include the N-RAS GTP/ GDP binding GTPases. Mutations in the *RAS* gene family are associated with constitutive activation of the RAS-mitogen activated protein kinase (MAPK) signalling pathway and are implicated in the pathogenesis of haematological malignancies. RAS mutations are described in 13-20% of adult AML and 15% of paediatric acute leukaemias. In addition, signalling from other transduction pathways of relevance to haematological malignancies is mediated via components of the RAS-MAPK pathway, for example, FLT3 and KIT. If farnesylation is essential for normal function of RAS, then inhibition of farnesylation of RAS proteins could block aberrantly activated RAS mediated pathways. A number of farnesyltransferase inhibitors (FTI) have been developed including lonofarnib and tipifarnib. FTIs are currently undergoing early Phase I and II studies in adult high risk leukaemias and myelodysplasias. Promising clinical responses are reported, however, they do not always correlate with decreased farnesylation of downstream proteins nor were activating RAS mutations predictive of response. Interestingly, a study of *in vitro* sensitivity reported tipifarnib sensitivity in paediatric AML and T-lineage ALL but not B-precursor ALL. Again, although RAS mutations were detected in 32% of AML samples and 18% of ALL samples in the study, they were not predictive of sensitivity to the FTI. Recently, the specificity of some FTIs for farnesyltransferase has been questioned, for example, the FTI BMS-1 has been shown to also target a related prenyltransferase Rab-geranlytransferase. A better understanding of the mode of action of FTIs would enhance the clinical development of FTIs, in particular, further characterisation of farnesylated proteins and their role in tumourogenesis and tumour progression would help towards more accurate biomarkers to predict FTI efficacy.

The targeting of a downstream component of an aberrantly activated signalling pathway is exemplified by the targeting of gamma secretase in the NOTCH signalling pathway. The NOTCH signalling pathway is an important developmental pathway in mammalian cells. Aberrations in this pathway have been specifically identified in T lineage leukaemias and lymphomas. Approximately 15-20% of childhood leukaemias are T lineage, and in around 1%, the t(7;9) chromosomal translocation has been identified. NOTCH signalling pathways involve the regulation of proteolysis and are implicated in normal early T cell development. NOTCH 1 constitutive activation is seen in T ALL with t(7;9). Proliferation of cell lines carrying NOTCH 1 'gain-of–function' alleles can be inhibited by inhibitors of the NOTCH1 signalling pathway. Mutations have been identified in the hetero-dimerisation domain and the PEST domains of NOTCH1. It is now recognised that 55-60% of paediatric T ALL carry these activating mutations regardless the presence of chromosomal translocation t(7;9). Physiological activation of NOTCH receptors occurs when ligands of the Delta-Serrate- Lag2 (DSL) family bind to the NOTCH extra-cellular compartment (NEC) subunit and initiate a cascade of proteolytic cleavages in the NOTCH transmembrane (NTM) subunit. The final proteolytic step is catalyzed by gamma secretase, which generates intracellular NOTCH (ICN). ICN translocates to the nucleus and forms a large transcriptional activation complex. Targeting the gamma secretase step provides a mechanism of downstream inhibition of the activated pathway. Gamma-secretase inhibitors were originally developed for the treatment of Alzheimer's disease. Gamma-secretase causes cleavage of beta-amyloid precursor protein and produces amyloidogenic peptides implicated in

the pathogenesis of Alzheimer's Disease. The usefulness of gamma secretases for this indication has been limited by the side effects, including inhibition of T and marginal B cell development and colon goblet cell metaplasia, however their short term, cyclical application may be of benefit in the treatment of T-cell malignancies. Currently, Adult Phase I and Phase II studies are underway, and the results are awaited with interest.

The immuno-phenotypic characterisation of leukaemias provides an ideal source of targets for antigen directed therapy. To date, gemtuzumab ozogamacin (GO. Mylotarg™) is the most developed antibody-drug conjugate undergoing clinical development in leukaemia. GO is an immuno-conjugate which links the recombinant humanised IgG4 antiCD33 monoclonal antibody to the DNA-binding agent calicheamicin. The CD33 antigen is a trans-membrane glycoprotein expressed on early multi-lineage haemopoietic progenitors and its expression diminishes with maturation and differentiation. Approximately 85-90% of childhood and adult AML cells express CD33. GO binds to CD33 and is internalised, where it releases the cytotoxic moiety calicheamicin. The clinical development of GO has focussed on the elderly AML population, where complete remission rates of 13% were achieved and led to FDA approval of GO in this patient group. It is recognised that the real value of GO will be in combination with other cytotoxic therapies and combination studies are on-going. GO has proved an excellent 'proof of principle' for antigen-targeted therapy and the rate of development of this field is accelerating. Exciting leukaemia specific antigen-directed therapies are in the pipeline, including antibody-mediated cellular toxicity and antibodies conjugated to radiation or other cytotoxic agents. Full evaluation of these agents in paediatric leukaemia is awaited.

Current approaches to the development of novel therapies in childhood leukaemia are driven by the accessibility of new agents for evaluation in Paediatric Phase I/II studies. In general, drug development is most commonly focussed on adult cancers and their potential efficacy in childhood malignancies is not considered. The rapid progress in understanding of the molecular basis of carcinogenesis and the cellular processes which maintain the malignant phenotype have led to the hope that we will be able to harness this knowledge in the development of more exquisitely targeted therapies, however, the molecular targets identified are not always as relevant in paediatric malignancies. Conversely, many molecular targets of specific relevance to paediatric malignancies are reported in the literature but, for a multitude of reasons, the majority are lost in the translation to a therapeutically useful target. Recent initiatives, in Europe (International Innovative Therapies for Childhood Cancer Group, ITCC. http://www.itccconsortium.org/) and in the US (Pediatric Pre-Clinical Testing Programme, PPTP, http://ctep.cancer.gov/forms/conceptprop.pdf) have been developed to systematically address this unmet need. Both initiatives are focussed on the evaluation novel agents in pre-clinical models of specific relevance to childhood malignancies, including childhood ALL. The number of paediatric patients eligible to participate in early clinical trials is fortuitously limited and accurate and relevant pre-clinical evaluation of new agents will provide an essential evidence-base for prioritisation for clinical development of these agents in paediatrics.

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SECTION B

The new international neuroblastoma risk group staging system – implications for surgeons

Tom Monclair

An obstacle to progress in clinical neuroblastoma research has been the lack of uniform criteria for diagnosis, staging, risk grouping and for determining response to therapy. To overcome this problem, a series of conferences have been held, resulting in the International Neuroblastoma Staging System (INSS), International Neuroblastoma Response Criteria and International Neuroblastoma Pathology Classification. Work is currently in progress designing International Neuroblastoma Risk Groups (INRG). The main intent with international uniform risk grouping is to facilitate comparison of results obtained with different treatment regimens. Since INSS is a surgico/pathologic system, it is NOT appropriate for assigning patients to risk-groups at diagnosis, before treatment. Today it is generally agreed that a patient's INSS stage may be determined by the initial treatment given. To illustrate the latter point let us consider three fully possible situations at the time of INSS staging for a patient when admitted to three different institutions: The hypothetical patient in question has a localised neuroblastoma crossing the midline and encasing one renal artery: In Institution A complete tumour excision was achieved by primary surgery. In Institution B the patient was left with a considerable macroscopic tumour residue after primary surgery, and in Institution C the patient had the entire tumour remaining after percutaneous biopsies. Correct INSS staging of this very same patient is Stage 1 in Institution A, Stage 2 in Institution B and Stage 3 in Institution C. Obviously stage, if used as a basis for risk grouping at the time of diagnosis, must be the same in all three institutions. Another limitation with INSS in this context is that stage 1 and 2 patients by definition have had their tumours excised at the time of staging. As a consequence patients that would have been staged as 1 or 2 if operated upon, cannot be properly staged if not operated upon. This will be the case for patients for whom a treatment policy of "wait and see" or "observation only" is applied. At an INRG conference in Whistler, Canada in September 2005, with 52 investigators from Australia/New Zealand, China, Europe, Japan and North America, a new staging system, the International Neuroblastoma Risk Group Staging System (INRGSS), was therefore designed to become one of the criteria used in the INRG classification system.

The INRGSS is based on radiological features, and localised disease is determined by the absence or presence of Image Defined Risk Factors (IDRF). The IDRFs are similar to the Surgical Risk Factors used in the LNESG1 study (Cecchetto G et al., J Clin Oncol 2005; 23: 8483-9). With modern techniques, imaging studies are considered less liable to subjective interpretation than the individual surgeon's assessment of resectability. Stored image studies can also be scrutinized in retrospect. They are therefore well suited for central reviews.

Surgical Risk Factors (=IDRFs) have been used for risk stratification in the SIOP Europe Neuroblastoma protocols for a decade. According to these protocols primary surgery is not recommended in presence of Surgical Risk Factors. It is therefore conceivable that colleagues used to work with Surgical Risk

Table 1: IDRF – Image Defined Risk Factors

- **Neck:**

  Tumour encasing carotid and/or vertebral artery and/or internal jugular vein

  Tumour extending to base of skull

- **Cervico-thoracic junction:**

  Tumour encasing brachial plexus roots

  Tumour encasing subclavian vessels and/or vertebral and/or carotid artery

  Tumour compressing the trachea

- **Thorax:**

  Tumour encasing the aorta and/or major branches

  Tumour compressing the trachea and/or principal bronchi

  Lower mediastinal tumour, infiltrating the costo-vertebral junction between T9 and T12

  Significant pleural effusion with or without presence of malignant cells

- **Thoraco-abdominal:**

  Tumour encasing the aorta and/or vena cava

- **Abdomen/pelvis:**

  Tumour infiltrating the porta hepatis

  Tumour infiltrating branches of the superior mesenteric artery at the mesenteric root

  Tumour encasing the origin of the coeliac axis, and/or of the superior mesenteric artery

  Tumour invading one or both renal pedicles

  Tumour encasing the aorta and/or vena cava

  Tumour encasing the iliac vessels

  Pelvic tumour crossing the sciatic notch

  Ascites with or without presence of malignant cells

- **Dumbbell tumours with symptoms of spinal cord compression:**

  Whatever the localisation

- **Involvement/infiltration of adjacent organs/structures:**

  Pericardium, diaphragm, kidney, liver, duodeno-pancreatic block, mesentery and others

Factors might associate IDRFs with treatment recommendations. However, to avoid misunderstanding, it must be emphasized that recommendation on treatment is NOT a part of the INRGSS, nor of the INRG. It must also be underlined that the list of IDRFs is designed for use at the time of diagnosis. The presence of IDRFs become irrelevant after preoperative chemotherapy.

Table 2: INRGSS – International Neuroblastoma Risk Group Staging System

Stage L1:	Locoregional tumour not involving vital structures as defined by the list of Image Defined Risk Factors
Stage L2:	Locoregional tumour with presence of one or more Image Defined Risk Factors
Stage M:	Distant metastatic disease (except Stage Ms)
Stage Ms:	Metastatic disease confined to skin and/or liver and/or bone marrow in children under the age of 18 months

Metastatic disease is defined as disseminated disease, i.e. not one continuous tumour. Involvement of non-regional lymph nodes, or discontinuous lymph nodes, are metastatic disease. However, an upper abdominal primary with lower mediastinal nodes and a pelvic tumour with inguinal nodes, are both locoregional diseases. A mediastinal tumour with pleural effusion and an abdominal tumour with ascites are both locoregional diseases. MIBG-scintigraphy is mandatory for INRG staging, and one definitive positive lesion at a distant site is sufficient to define metastatic disease. Bone scintigraphy is needed if negative MIBG (i.e. tumour is not MIBG-avid or the primary has been removed before MIBG-scintigraphy). Bone marrow disease is determined by morphology; not immuno-cytochemistry or molecular studies. A cut-off age of 18 months was chosen for INRGSS Stage Ms, which otherwise correspond to INSS Stage 4S. This decision is based on statistical analyses which show that the beneficial effect of young age extend beyond infancy.

The midline is NOT included in the staging criteria in the INRGSS. The "midline"-dilemma is covered by the IDRF definitions. Nor is the lymph node status included in the staging of localised disease. Accurate lymph node assessment requires operation, and many patients have not been operated upon at diagnosis. The assessment of lymph node involvement has been a problem for surgeons even after surgery. For example: not everyone is aware of the fact that a lymph node located between the aorta and the inferior vena cava is ipsilateral to a rightsided and contralateral to a leftsided tumour.

Surgery is not required for INRGSS staging. For risk grouping according to INRG, however, the biological features of the tumour must be known. For that purpose tumour excision, or at least a biopsy, is required. The Whistler conferees decided that **INRGSS** together with **Age**, **MYCN Status** and **Histology** should be used for defining the INRG. Additional statistical analyses are being performed to determine if other factors should be included in the INRG.

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The Relevance of Nutrition to Pediatric Oncology

Paul C Rogers

Nutrition is an under acknowledged topic within paediatric oncology. Nutrition is relevant to most of the components within the broad meaning of cancer control. These include epidemiology, prevention, biology and pathogenesis of cancer in children, treatment of the cancer and obviously supportive care during cancer treatment. This presentation will attempt to highlight why we need to think of nutrition as a fundamental aspect of cancer control and raise questions that we paediatric oncologists need to address.

There is minimal research undertaken on the epidemiology and potential prevention of childhood cancers from the perspective of malnutrition during preconception, pregnancy and the different phases of childhood growth & development. Folic supplementation during pregnancy has been associated with a decreased risk of acute lymphoblastic leukemia. There is possibly an association of folic acid deficiency with neuroblastoma and brain tumors. Mechanisms that could explain association between folic acid deficiency and increased cancer risk are changes in DNA methylation and impaired DNA synthesis and repair. It is probable that there maybe other nutrient deficiencies during pregnancy and early childhood development leading to gene pathway deregulation. Impaired nutrition is causatively involved in many other diseases in childhood and subsequently in adulthood. Cancer is one of those pathological conditions related to impaired nutrition in adults but poorly understood for pediatric malignancies. In adults the epidemiological evidence for nutrition related cancers is growing and potential preventative strategy for adult cancers should begin in childhood. We need to focus on the nutrition of the mother, fetus, neonate and the young child and the potential impact of nutrition on the life long risk of cancer. The World Cancer Research Fund UK has set their research priorities as follows

- Studies that focus on nutrition of the mother, fetus, neonate and young child and lifelong risk of cancer
- Studies investigating energy intake and expenditure, body composition, body weight and cancer risk
- Studies investigating the role of physical activity /inactivity and cancer risk
- Projects focusing on countries in transition from developing to developed economic status in order to study the relationship between food, nutrition, physical activity and cancer.
- Studies investigating the interventions aimed at changing eating habits, physical activity or nutrition status at individual, community or population levels.

These population based research questions need to be embraced by paediatricians.

The understanding of the biology of pediatric malignancies is rapidly evolving. Nutrition may have an effect on the biochemical and gene signalling pathways that are relevant to promoting or inhibiting cancer development. Research into the effects of nutrients on the expression of specific genes associated with apopotosis, cell proliferation, angiogenesis, adhesions molecules, cytokines and cytokine receptor expression is taking place. Animal models have shown that changes in diet can affect DNA methylation. Dietary supplementation of the glutathione system leads to a diminution of reactive oxygen species and the down regulation of the NF-kappaB which confers a variety of benefits including inhibition of pro-inflammatory cytokines and sensitisation of cancer cells to chemotherapy. These are exciting developments that may well have practical relevance.

As paediatricians we recognise the importance of good nutrition on normal growth and development and the maintenance of normal physiological homeostasis. There are clearly described pathological disorders that are evident in nutritional deficiencies such as protein calorie malnutrition, vitamin deficiencies and trace element deficiencies. Oncogenesis is highly complex and the hypothesis that the initiation, promotion, and progression of oncological disease in a growing child may be influenced by the nutritional state at preconception, gestation, neonatal and periods of growth is plausible. The concept definitely needs further research.

There is an association between nutrition and immune status. There is a growing field of immune nutrition that is investigating functional foods or nutraceuticals that may influence immunological response mechanisms. Functional foods are foods containing natural, bioactive chemical compounds that have health promoting, disease preventing or medicinal properties (polyphenols, phytoestrogens, fish oils, carotenoids, phytosterols, soy isoflavones, vitamins and trace elements). Nutraceuticals are products that are isolated or purified from functional foods and prepared in pharmaceutical forms (glutamine, omega-3 fatty acids, arginine, and ribonucleic acids, N-acetylcysteine). Immunonutrients may act as pharmacologic agents since these nutritive agents have the potential to modulate the immune response at a molecular level and thus their effects may be disease-state specific**.** Evaluating single nutrients in the same methodology as small molecule drugs is probably flawed as nutrients rarely function in isolation. The immunonutrition approach may well have relevance to cancer prevention, cancer therapy and supportive care during therapy.

The prevalence of malnutrition as defined by protein calorie malnutrition (PCM) ranges from 50-60% depending on diagnoses, stage, treatment and social economical status. Nutritional status at diagnosis and its relevance to overall survival and disease outcome is controversial. There are reports that under nutrition is associated with poorer survival especially in countries where malnutrition is prevalent. In developed countries this does not always appear to be a prognostic factor. Malnutrition does appear to be associated with impaired intolerance to chemotherapy, impaired immune status with increased risk complications to therapy such as infection, marrow suppression and altered treatment schedules. Improving nutritional status does enhance the feeling of well being. The pharmokinetics of drugs are affected by PEM with decreased clearance and alternate distribution of drugs. This has been well described for Methotrexate.

A recent view of the Children's Oncology Group nutrition committee reported a wide disparity of nutritional practice in the assessment and nutritional intervention for children on therapy. This report showed that assessment of nutritional status does not occur on a consistent basis and when it does it is mostly based on weight change alone. Consistency is required for the assessment of nutritional status, categorization thereof and the use of clinical pathways for intervention. While the impact of nutritional intervention in children with cancer is uncertain with respect to the cancer outcome, we as pediatricians accept that adequate nutrition is essential for growth, development and well being of children without disease. Thus it seems prudent and rational that adequate nutrition should be ensured for children with cancer.

Cancer cachexia is multifactorial both at the time of diagnosis and during treatment. It is critical to identify the interacting factors of host, tumor, treatment and complications thereof which may be contributing to malnutrition so that the appropriate interventions can be employed that may ameliorate some of those factors. While some of the contributing factors are recognized for cancer cachexia, many of the mechanisms are poorly understood and may well be different in the growing and developing pediatric patient compared with adult patients. Animal studies and the adult literature indicate pro inflammatory processes are implicated in the hypermetabolism and weight loss associated with cancer associated cachexia

Nutrition assessment of the pediatric cancer patient is similar to the assessment of the nutritional status of any other pediatric patient. Asseassments should be longitudinal so that trends can be ascertained. Practically, weight is the baseline measurement most frequently

undertaken and followed. Assessments based on weight alone can be misleading, especially in the acutely ill patient when fluid balance may be disturbed, or the presence of edema or mass disease. Additionally, weight may be maintained but lean body mass can be diminished. This can be the case in the patient who is obese at the onset of treatment. Weight for height is a widely used assessment of PEM. Body mass index (BMI) is considered a more reasonable parameter for assessment over the age of 2 years and ideal body weight (IBW) under the age of 2. BMI has to be interpreted relative to population reference data as BMI changes with age and differs between genders. It is considered a better proxy for body fat and lean body mass. It is the most appropriate index in assessing the other end of malnutrition i.e. obesity. Health care providers should measure and plot on the appropriate population charts, weight for age, height for age, weight for height, BMI, head circumference (under the age of three years) and mid arm circumference to better assess muscle wasting and triceps skinfold for estimation of adipose tissue. Evaluation of nutritional intake is a necessary component to try and predict those patients who are at risk of becoming undernourished. Biochemical measurements are of some use but interpretation can be difficult due to confounding factors such as sepsis and acute phase reactants during sepsis.

Cytotoxic therapy and sepsis may deplete the body of micronutrients. Decreased antioxidant levels have been reported following chemotherapy and trace elements may also be depleted, especially Zinc.

Nutritional intervention should be an active and continuous aspect of supportive care to sustain growth and development, plus improve well being and quality of life. Dietary intervention is the most commonly commenced if there is a weight loss, despite the limitation of weight being a sole criterion. A proactive approach is to identify patients that are at risk for malnutrition and intervene before there is significant weight loss and/or loss of lean body mass. Increasing oral intake should be the first approach. There is a wide variety of oral supplements available to increase calorie and/or protein intake. Enteral feeding can be increased by the use of tube feeding which may either be nasogastric or nasoduodenal, or the placement of percutaneous gastrostomy, duodenostomy or enterostomy tube. Many preconceptions exist about nasogastric (NG) tube feeding. There is a reluctance to place a NG tube as this is often seen as a punishment by the patients. NG tubes are frequently avoided in the neutropenic or thrombocytopenic patients, presence of mucositis, plus the concern of aspiration or sinusitis. These are all cited as reasons against the use of a NG tube. There is insufficient evidence either to support or dispute these perceptions. What is well described is the importance of maintaining gut integrity and the use of enteral trophic feeds are encouraged.

Specific designer nutritional supplements are available but further evidence is required before their routine use can be recommended. The Children's Oncology Group Nutritional committee undertook a pilot study for patients on chemotherapy using an undenatured whey protein to enhance Cystine delivery, which is a precursor for glutathione production. The pilot study showed an increase of glutathione over the base level and weight gain in the majority of patients. A randomised study has been proposed. Glutamine supplements have been advocated to improve mucosal turnover, especially in stem cell transplant patients. The value of a neutropenic diet is uncertain but there is some evidence to indicate that it is of no value. Appetite stimulants are used but remain controversial.

Parenteral nutrition (PN) should be considered when all attempts for sufficient enteral feeding have failed or contraindicated such as presence of neutropenic entrocolitis (typhlitis) or gut that is not functional. PN is clearly associated with a significant increased risk of infection, hepatotoxicity and variety of metabolic abnormalities. The utility of PN has been best defined in stem cell transplant patients who have prolonged gut damage due to the preparative regimen, Graft-Versus-Host Disease and/or gut infection. If PN is required, when possible, minimal enteral feeding should continue to preserve gut integrity and function.

Nutritional supportive care for pediatric oncological patients should be undertaken with the same diligence as one does for other supportive care issues.

Obesity

In North America thirty percent of children over age six have a BMI greater than the 85% percentile. Pediatric Oncology patients have not been spared and reported in long-term follows up studies. The metabolic syndrome of impaired glucose tolerance, diabetes mellitus, insulin resistance, hypercholesterolemia, hypertension and cardiovascular disease is now being documented in children. Obese children tend to become obese adults. There is increased risk of the adult obese patient developing cancer and obese adult patients have a poorer outcome. Obese paediatric patients with AML or ALL have a poorer outcome. An important aspect of obesity in children is the potential impact on the pharmacodynamics and pharmacokinetics of cytotoxic agents that may be altered in obese patients. Appropriate dosing for the overweight patient is unknown and does need further evaluation. Obesity is not only due to inappropriate nutrition but overall lifestyle. Obesity intervention is about prevention of which monitoring nutritional intake of both quantity and quality should be part of our every day care.

Conclusion

Nutrition and the overall impact on our patients deserves greater attention than is currently given. Such a fundamental component of life needs greater emphasis in our day to day care of our patients as well as our clinical and basic research programs.

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The expanded future of pediatric radiation oncology

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The new technologies in oncologic imaging and radiation therapy are designed to improve local-regional control, reduce treatment related morbidity, and are expected to bring about higher cure rates with improved quality of life. While these enhancements in tumor localization and radiation delivery are not age specific, they offer significant advantages to children and adolescents in whom early local tumor control is critical, and in whom late effects of cancer therapy can be severe.

Local failure accounts for the primary obstacle to cure in children with primary central nervous system tumors, intraocular tumors, bone and soft tissue sarcomas, and many other solid tumors [1, 2]. Radiation therapy is an effective modality to achieving local control, but high radiation doses to large treatment volumes, as delivered in the past, have been associated with significant late effects which, for some, have limited its effective use. The late effects of cancer therapy, as given several decades ago, are now being recognized. They include: impairment of soft tissue and bone growth; organ dysfunction including cardiac, pulmonary, gastro-intestinal, and renal; gonadal effects with potential infertility; endocrine sequelae; hearing and/or visual dysfunction; neuro-cognitive effects; psychosocial issues, and the induction of second tumors. Among these, secondary carcinogenesis and potential organ dysfunction are some of the most severe of the late effects from radiation therapy. The Childhood Cancer Survivor Study (CCSS) now has data from children who are at least 5-year survivors of their malignant disease, which report an approximate 5% cumulative incidence of second and subsequent cancers at 25 years following the diagnosis of the first cancer, and this incidence continues to rise. The large majority of these second malignancies occur in patients who received radiation as curative treatment for their first malignancy, with an approximate 6.6% cumulative incidence for those irradiated, as compared to an approximately 3.8% cumulative incidence for those non-irradiated, at 25 year follow-up [3,4]. Among these secondary malignancies, breast cancer in Hodgkin's disease and sarcoma survivors is most prevalent [5]. In the CCSS study of long term survivors, girls receiving chest radiation for treatment of Hodgkin's disease had a standardized incidence ratio (SIR) or relative risk of 26.3 of developing breast cancer. In addition, there is a dose related incidence of central nervous system glioma and meningioma after the diagnosis of a first brain tumor [6]. The dose-response for the excess relative risk (ERR) of a brain tumor is estimated to be 0.33/Gy for glioma and 1.06/Gy for meningioma. For glioma, the ERR/Gy is highest among those exposed at ages under 5 years. The excess of glioma and meningioma is concentrated among children with leukemia or CNS cancer as their initial cancer. The standardized incidence ratio (SIR) for glioma is 8.7 overall, and the excess absolute risk (EAR) is 19.3 per 10,000 persons per year. The higher risk of subsequent glioma in children irradiated at a very young age may reflect greater susceptibility of the developing brain. . However secondary thyroid cancers are not dose related beyond approximately 10 Gy. There is a linear relationship from about 0.05Gy to 0.1 Gy to 5Gy to 10 Gy, with an ERR of 7.7/Gy in thyroid cancer. The EAR is 4-5 thyroid cancers per 10,000 persons per year per Gy. [7]. Organ dysfunction is also being recognized, but here the contribution from radiation is often difficult to separate from that of chemotherapy. It is important to remember that the serious late effects data, which impact all current treatment decisions, are reported among the cohort of children who were effectively treated for their first malignancy, often times with radiation, and who have survived long enough to suffer another

malignancy. These data usually do not account for genetic influences in carcinogenesis, an increasingly important predisposing factor for all [8, 9].

Standard of care today using radiation therapy for children and adolescents includes risk-adapted protocols, which recommend 3-dimensional conformal techniques when treating with curative intent [10]. This therapy provides greatly improved radiation distribution over treatments of the past decades. Improved tumor localization and image acquisition using PET/CT as well as MR/CT fusion into treatment planning systems facilitate the planning and delivery of radiation. Cone beam CT and fluoroscopic imaging assist the verification and accuracy of these complex multi-dimensional systems. In select tumors, biologic and molecular imaging and treatment hold additional promise, and may be incorporated into specific treatment protocols.

Many centers are further investigating the use of very highly conformal radiation beams for specific anatomic sites such as delivered by intensity modulated radiation therapy (IMRT), stereotactic radiosurgery (SRS) and stereotactic radiation therapy (SRT), frame based or frameless [11-13]. Brachytherapy for specific malignancies and anatomic sites has demonstrated utility in children and adolescents (14). Intraoperative radiation therapy (IORT) is useful in select situations [15-17]. Targeted therapy with a bone-seeking radiopharmaceutical such as samarium-153 is being investigated in the treatment of osteosarcoma and bone metastases, and may have usefulness if the pancytopenia can be addressed with stem cell infusion [18-19]. Image guided radiotherapy (IGRT) using 4-dimensional radiation therapy, respiratory gaiting, on-board imaging and tumor tracking, accounting for organ motion during treatment, is now being pioneered for specialized anatomic sites. Conformal proton beams and intensity modulated proton beams are planned in a few centers and are hoped to further enhance dose distribution, particularly for children with solid tumors [20-22]. It is hoped that these highly conformal treatment fields will reduced late effects, specifically radiation-induced second cancers [23].

These new treatment techniques offer enormous potential for children and adolescents in whom the avoidance of normal tissue toxicity is an essential endpoint. However, they require close interaction between radiation oncologist, physicist, and biologist for complex technologic advances and biologic endpoints. These new technologies and research advances, being made in large cancer centers, currently apply to a disappointingly small number of children afflicted with malignant disease. From a global perspective, the number of children worldwide who will profit from these highly specialized technical advances will be just a small fraction of the world's pediatric cancer problem. Nevertheless, these advances are an essential research step, as new technology offering improvement for a small proportion of children will advance the biology, physics, clinical research and patient care for all children with malignancy. During the past year reports have been published which demonstrate that in countries with limited, even very limited resources, more children with cancer have been cured than previously [24-25]. However access to radiation therapy is very limited for the majority of children. In many countries, it is only in private institutions where one has access to radiotherapy, and it is limited to those who can afford the treatment. It has been estimated that <1% of all children who might benefit from radiation therapy actually receive this therapy. So, there are several issues of concern: one is limited availability world wide, and another is limited access even in developed countries. If even a few children with non-resectable, chemotherapy resistant, solid tumors can be effectively treated by these new treatment techniques, we will see further increases in the total numbers of children who will be cured of their cancer. However, the ultimate value of new technology in radiation therapy will require controlled clinical trials and cost effective analyses to define its use and applicability.

The possibility of cure without late effects is now a probability for those children and adolescents undergoing treatment with radiation. The vision of the expanded future for pediatric cancer confirms that never before has there been a more exciting time for pediatric radiation oncology than the present.

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Hemophagocytic lymphohistiocytosis A challenge for the pediatric hematologist-oncologist

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Hemophagocytic lymphohistiocytosis (HLH) is a genetically heterogeneous disorder characterized by an hyper-inflammatory syndrome with fever, hepato-splenomegaly, cytopenia and, less frequently, central nervous system involvement. Frequent alterations include low levels of fibrinogen, high levels of ferritin, triglycerides, and the ? α-chain of the soluble interleukin-2 receptor (sCD25).[1-3] Since symptoms may resemble those of leukemia or lymphoma, patients will be referred to pediatric haematology-oncology units, and bone marrow aspiration is usually performed early during the diagnostic work-up, showing — at first or at repeated evaluations during the disease course — hemophagocytosis by activated macrophages.

The natural course of HLH is rapidly fatal within a few weeks in the majority of cases unless appropriate treatment, including corticosteroids, cyclosporine, and etoposide result in transient disease control.[3-6] So far, only patients who underwent hematopoietic stem cell transplant have been cured.[7-9]

Differential diagnosis of HLH may be difficult.[10] When HLH is diagnosed according to current criteria,[11] the main duty of the pediatric haematologist-oncologist is to try to put the patient in a more stable condition, since this disease still has a relatively high risk of rapid fatal progression within a few weeks when unrecognized, occasionally even despite chemo-immunotherapy. Once initial disease control has been achieved, the main target is to try to understand why the patient developed HLH, a clinical syndrome characterized by macrophage hyperactivation. This may occur due to several reasons: concurrent acquired immune deficiency (chemotherapy, immunosupression following organ transplant) or infection (EBV, Leishmania, bacteria..) or autoimmune systemic disease (juvenile arthritis, lupus) which may be even heralded by HLH. Otherwise, a previously healthy infant may present with HLH as the initial manifestation of a constitutional immune defect. In such cases, report of consanguinity or undiagnosed fatalities in siblings may direct the diagnostic work-up toward a congenital immune deficiency.

Demonstration of frequent association with common pathogens and evidence of impaired natural killer cytotoxic activity provided the rational for considering HLH as a selective immune deficiency.[12-14]

Linkage analysis led to identification of an association between FHL and the genomic region 9q21.3-22 (FHL1, MIM 603552), where the gene responsible for the disease was not yet identified. A simultaneous report established a linkage with another region, 10q21-22, in which the perforin (*PRF1*) gene was identified as responsible for a wide proportion of cases of FHL (FHL2, MIM 603553).[15] In patients with FHL2, *PRF1* mutations induce a complete or partial reduction of the synthesis of the perforin protein. As a result, the cytotoxic machinery of NK cells is markedly impaired.[16] Since a formal genotype-phenotype study is not yet available, the exact contribution of the different mutations reported in the literature is not yet clear, although some linkage between a few individual mutations and particular ethnic groups appear to be preliminarily defined.[20-22] In 2003 a third locus, 17q25, was reported in linkage with FHL (FHL3, MIM 608898).[23] The product of the involved gene, the Munc13-4 protein, is thought to contribute to the priming of the secretory granules before they fuse into the plasma cell membrane. Mutations in this gene impair the delivery of the effector proteins, perforin and granzymes, into the target cells resulting in defective cellular cytotoxicity and a clinical picture which appears identical to that associated with *PRF1* mutations.

Very recently, on the basis of a genome-wide screening, a fourth chromosomal region (6q24) has been reported in linkage with FHL in a subset of Kurdish patients (FHL4).[24] Mutations of the syntaxin 11 gene, mapped in this region, are thought to alter intracellular vesicle trafficking of the phagocytic system.

Based on the current knowledge, *PRF1* mutations account for about 40% of cases of FHL (type 2), and mutations of the *Munc13-4* gene for an additional 30% (type 3). This last subtype may present at any age including young adult, often with major CNS involvement, and is usually associated with a marked defect of NK activity.[25]

At present the clinical approach to patients diagnosed with HLH includes evaluation of NK activity and flowcytometric analysis of intracytoplasmic perforin. Most of patients who lack perforin expression will show *PRF1* mutations. Oculo-cutaneous albinism will address to one of the few syndromes with immune deficiency.[26] For the remaining patients mutation analysis of the Munc13-4 gene will be the next step, since about 30% of patients will be linked to this gene.

Current research in this field is aimed at identification of the genetic defect hopefully in far most of FHL families. Although expensive and time consuming, it is mandatory to confirm the diagnosis, to refine the therapeutic choice including indication to hematopoietic stem cell transplant, to identify the carriers and thus select familial donors. Information on the familial genetic marker may allow also prenatal diagnosis. Identification of pre-symptomatic affected siblings may represent another challenge for the medical team, which will provide a tailored clinical and functional monitoring to determine if and when treatment may be indicated. In the minority of families in which none of the available markers is helpful, analysis of putative genes and linkage analysis of consanguineous families may provide novel insights.

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Understanding experiences of childhood cancer: a fresh perspective

Mary Dixon-Woods

In this lecture I aim to show how study of experiences of childhood cancer can benefit from a multidisciplinary approach that recognises the contribution of the range of social sciences, and that is informed by a sound understanding of the clinical issues in the diagnosis and management of childhood cancer. There are important reasons for revisiting the social science study of childhood cancer at the present time. First, a well-founded social science perspective on childhood cancer is necessary not only for researchers, but also to inform policy and practice in relation to the care of children with cancer and their families. However, some of the most important contributions to the field (e.g. Bluebond-Langner's classic study of the private world of dying children, published in 1978) were written during a period when childhood cancer had poor outcomes, and a majority of children died of the disease. Now, most children survive, and questions must now focus on *living with* and *surviving* childhood cancer.

Second, the social science study of childhood in general is burgeoning (James et al 1998; Jenks 1996; Lee 2001;Mayall 2002), with the emergence in the last two decades of a "new" sociology childhood. This has taken the route of distinguishing how a sociology of childhood might differ from a psychology of childhood, and it has been successful in identifying previously ignored questions and encouraging the application of methods consistent with those questions. With the development of the wider field of childhood studies there are opportunities for refreshing and deepening our understanding of experiences of childhood cancer. However, it is also important to offer a critique of some currently dominant approaches within childhood studies, particularly those informed by a libertarian perspective.

Third, there is now a substantial body of empirical research on experiences of childhood cancer. Though the quality of this work is variable, it is important to begin to synthesise it to begin to produce an integrated and holistic account of the evidence, to identify where the gaps might lie, and to identify an agenda and directions for future research. It is also appropriate to synthesise the work on childhood cancer with empirical and theoretical work in other fields. For example, the sociology of adult illness has much to offer in terms of a theoretical approach to experiences of serious chronic disease, particularly in the absence of a more fully elaborated sociology of childhood illness.

I will argue that it is increasingly important that social science accounts of children's experiences must go beyond assessments of psychological morbidity. Children and young people with cancer should be taken seriously as social agents in their own right, and be seen as active in giving meaning to their own experiences. They are also active participants in the emotional, biographical, and physical work of having childhood cancer, including its strategic management. I will also suggest, however, that some recent libertarian arguments about the potential role of children and young people in decision-making go too far.

I will suggest, for example, that the insistence that there must be "open communication" with children, and that children should be involved in decisions about and able to consent or refuse medical treatments, has very serious implications. I stress the inappropriateness of adopting inflexible and rigid approaches to communication (whether in the direction of full disclosure or the reverse), particularly in the striking absence of research about the priorities and preferences of children in this sensitive and difficult area. The potential consequences of

open communication and involvement in decision-making are largely unknown, and there are many uncertainties about how disclosures of information and children's expressed views should be managed. How seriously the objection of a very ill child to being subject to a painful, frightening or distressing but necessary procedure should be taken into account needs to be considered within this very specific context. To argue that the often agonised efforts of parents and staff to gain children's cooperation are simply the actions of an oppressive majority is to risk offering a crude and simplistic account of a highly complex situation. Similarly, to argue that information is withheld from children through taking advantage of an unequal balance of power coupled with the paternalistic instincts of adults does not do full justice to the complexities of situations in childhood cancer and the positioning of the parties involved. For example, there is a need to recognise the ways in which children are strategic in their use of silence in consultations, and in their use of parents as envoys and communication buffers. I propose that imposing certain forms of uniform "rights" on children in relation to information and decision-making is in fact potentially oppressive (effectively functioning as a prior determination of their best interests).

Much more needs to be done to explore the extent to which (individual) children really wish to be decisive and the long term outcomes of those decisions, their preferences regarding the form of their relationships with professionals, and how their needs, including emotional and informational needs, can be better met. In this, there is a need to be clear that some issues are not easily tractable; once the explanation that it is not simply lack of will that holds adults back from sharing information and decisions with children falls away, other explanations demand far more complex and demanding solutions.

These issues highlight the need for theorising of childhood illness to take account of the science of development, to avoid, for example, confounding the general social competence of children with specific competences in medical decision-making. Here, the damaging consequences of the caricaturing of developmental psychology that has dominated some recent work in childhood studies is most apparent. Developmental psychology is much more sophisticated and diverse than its critics have given it credit for, and has a vital role to play in informing not only debates about the characteristic cognitive processes of children at different ages, but also in the assessment of individual competences. Study of these important aspects of childhood cancer is likely to involve approaches that integrate contributions from sociology and psychology, and that use methods including ethnography and conversation analysis as well as directly accessing the views of children and their families. The roles of health professionals other than doctors will be a key interest of this future work.

I will argue that social science inquiry into the nature of parents' and families' experiences must also go beyond a narrow focus on psychological morbidity. The experiences of parents are especially important and interesting, though attention also needs to be given to siblings and other socially adjacent people. Lupton (1997) describes how parents have acquired a whole series of obligations in relation to their children, particularly in relation to their health and welfare. Childhood cancer serves as a particular form of the intensification of parenthood. Parenting a child with cancer needs to be re-characterised to draw attention to how parents' identities and social obligations position them in relation to the medical world, to highlight the emotional work carried by parents, and to show how becoming and being a parent of a child with cancer invites surveillance of parenthood. I am conscious, of course, that most research has focused on accounts of mothers, and that there is risk that research could (unwittingly) reinforce aspects of motherhood as "natural" and non-negotiable, rather than reflexively produced in response to dominant social and discursive constructions. Clearly the ways in which family members come to recognise and adopt norms and roles in relation to childhood cancer will be an important aspect of future research. So too will be exploration of different forms of families, including children who are in the care of social services who whose parents are unable or unwilling to accept the roles that are socially prescribed for them.

I will illustrate many of the points raised in this lecture with reference to a series of empirical studies in which I have been involved, including in particular a project funded by the UK Economic and Research Council's Science in Society Programme on childhood cancer tumour banking. Using in-depth interviews with 81 parents and children, I will show how we need a sophisticated and theoretically rigorous approach to understanding issues of information, sharing decision-making, and consent.

Many of the issues I raise reflect the absence of a properly elaborated sociology of childhood illness to complement the psychology of childhood illness. Current social science work in childhood cancer is of variable quality, and much of it is disappointing. This poses major challenges for those attempting to theorise in a specific area such as childhood cancer. There is an urgent need to begin to distinguish in what ways a sociology of childhood illness might draw on constructs already developed within the sociology of adult chronic illness, and to identify where completely new forms of theorising are required. There is some evidence that many existing constructs have considerable explanatory value as far as experiences of chronic childhood illness is concerned, but much more needs to be done to explore and develop these and others. In taking the specific field of childhood cancer forward, it is clear that there is a particular need to attend to the different sub-groups. Different forms of tumours may have very different effects, and there is a real need establish the extent to which they should be seen as different diseases. Much more, too, needs to be done to understand the experiences of children of different ages. I suggest that much more thorough, rigorous, and sophisticated empirical research and theorisation is required. An interpretive interdisciplinary approach is likely to offer the most fruitful way forward.

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Pediatric Hematology/Oncology in Morocco

Mhamed Harif

The Kingdom of Morocco, situated at the North West tip of Africa and bordered by the Atlantic Ocean and the Mediterranean Sea, covers an area of 710,850 km^2. The population exceeds 30 million, children under the age of 15 representing 31 % of the population. Morocco faces the problems typical of developing countries; 19% of the population living below the poverty line and up to 50% being illiterate. Only 16% of the population has health care insurance.

The health care system in Morocco is a mixture of government institutions as well as private system. Most of economic activity is concentrated in Casablanca and Rabat (the capital).

Organization of care

Up to late seventies of the last century, patients with cancer have been treated in a radiation center in Casablanca. This center was not equipped to take care of children with cancer. Patients were admitted in different wards of the hospitals and treatment programs varied greatly according to availability of drugs. The tremendous progress reported in childhood cancer stimulated the creation of specialized units.

Organized pediatric oncology activity started in the early 80th when two units opened in the two university hospitals of Morocco, Casablanca and Rabat. In Casablanca the Department of Hematology and Pediatric Hemato-Oncology was created in 1980 by Noufissa Benchemsi and Said Benchekroun. In Rabat, Fouzia Msefer-Alaoui initiated a unit in Children's Hospital in 1982.

The two teams tried to apply modern protocols. There has been good pediatric surgery, pathology, radiology teams that helped the young pediatric oncology teams. At that time, many medications were not available.

In 1986, the National Cancer Institute opened in Rabat, which offered a better radiotherapy facility for children. Another unit opened in Casablanca and other private clinics in Casablanca and Rabat started a consistent pediatric oncology activity.

Given the inadequate quality of supportive care facilities, specifically the lack of blood products and antibiotics, intensive chemotherapy was not used. Most patients had no health insurance and could not afford to pay for their treatment and not infrequently even the cost of transportation to the units. Likewise, the hospital had very limited resources and could not provide such expensive treatment. It thus became clear that quality of care could not improve without additional help. Associations of volunteers and donors were therefore set up. Agir in Casablanca (www.association-agir.org) and Avenir in Rabat (www.almoustakbal.org) have proved to be very efficient and have helped the hospital teams in their endeavors to provide optimal care. Most of patients needs regarding medication is provided by these NGOs. These associations have also renovated the wards and hired personnel to help the teams. In Rabat, Avenir constructed a house for the parents and patients named Maison de l'Avenir. In Casablanca, Agir set up bone morrow transplantation unit, the first one in the country.

Supportive Care

Infection related mortality of patients was of great concern. From 1980 to 1985, retrospective analysis of 138 neutropenic patient's mortality was of 36% [(1)]. No curative treatment was proposed for patients with acute non lymphoblastic leukemia (ANLL) in the first years. There have been special interests in improvements in supportive care, increased awareness of the importance of hygiene, education of patients and improvements in nursing education. Another factor has been the

quality of blood products: a Quality Control Committee for Blood Transfusion Services has been set up. Screening for hepatitis B and C and for HIV is now routinely carried out. As a result, the incidence of hepatitis C has fallen from 26% to 6% in patients receiving multiple transfusions. Infection Control Committee has been set up and is working closely with the medical staff.

Improvements in supportive care have resulted in a significant decrease in mortality from neutropenic sepsis. From 1983 to 1995, 117 patients with ANLL have received 2 to 3 courses of chemotherapy using daunorubicin or doxorubicin and standard dose cytarabin. Complete remission (CR) was obtained in 57% of patients and toxicity related death was of 18.7% [2]. From 1996 to 2003, 67 patients received 2 to courses of chemotherapy one course of high dose cytarabin. The CR increased to 69% and toxic death t dropped to 11%. This coincided with the renovation of the ward and better equipment of patients' rooms (including sinks). EFS in this group is 28%.

Lymphomas

In view of the potentially good prognosis of patients with lymphoma, this was considered to be a priority and resources were allocated to improve survival in these patients. Ninety five patients with Burkitt's lymphoma have been treated according to the French LMB89 protocol. Most patients (73.5%) had an abdominal presentation; the diagnosis was made on the basis of fine needle aspiration in 60% of cases. The majority of cases (63%) had Stage III disease. Complete remission was achieved in 68.5% of cases; the 5 year survival was 56%. Ten of the 15 patients who died did so before or shortly after initiation of treatment due to metabolic and nutritional complications [3]. The greatest problem is that patients present with very advanced disease because of delays in diagnosis and the long distances involved in reaching the hospital.

With regard to Hodgkin's disease (HD), 181 children have been treated up to 2001. Chemotherapy regimens comprised 'MOPP', 'COPP' or 'ABV'. Again, most patients presented with advanced stage disease and the high rate of lost to follow-up (35%) because of the unaffordable cost of work-up and treatment. An unexpected finding was the incidence of Hodgkin's disease in very young children at the time of presentation [4].

Collaborative programs

The teams are now well established and are treating more that 800 new patients a year. A good multidisciplinary group of pediatric oncologists, surgeons, pediatric radiologists, pathologists and radiation therapists are working closely together. This is reflected in some good results obtained in solid tumors [5]. The teams are working closely together and created the Moroccan Society of Hematology and Pediatric Oncology (SMHOP) in 1996. SMHOP (www.smhop.info) has organized continental meetings of SIOP in 1998 and in 2006 and is organizing regular national meetings.

Moroccan group has developed a fruitful cooperation with International Outreach Program (IOP) of St Jude Children's Research Hospital (Memphis, TN, USA) directed by Judith Wilimas and Raul Ribeiro. The program has focused on nursing, together with improvement in pathology services and infection control, as well as data management, immunophenotyping and cytogenetics for leukemia and the development of adapted therapeutic protocols. Internet based communication system is being used as a useful tool to develop various actions and discuss patients cases to have second opinions. With the help of IOP, Moroccan group has developed protocols for ANLL (AML-Ma 2003) and HD (MDH-Ma 2004). In AML-Ma 2003 the objectives are to get CR rates at 70%, toxic deaths less that 10% and EFS at 40%. The preliminary data on the first 27 patients included showed that CR is at 85%; toxic deaths at 11%. It is interesting to note that the toxicity is the same as in the earlier experience even with more intensive treatment. In MDH-Ma 2004, the main objective is to reduce the abandonment rate to less than 10%. Of the 51 patients included so far, the abandonment rate is of 10% but the initial workup is still lasting 3 weeks.

Moroccan group is also an active member in French African Pediatric Oncology Group (FAPOG) led by Jean Lemerle (Villejuif, France). This group is now focusing on Burkitt's lymphoma and Wilms tumors. Interesting results have been achieved through a prospective

multicentric work in 6 African countries (Algeria, Cameroon, Madagascar, Morocco, Senegal, and Tunisia) [6, 7].

More recently, Moroccan group has been selected among the country benefiting from UICC My Child Matters initiative [8]. Pain control and early diagnosis in childhood cancers are being developed through this initiative.

What next?

The medical teams are very dedicated and enthusiastic. They are still striving to diagnose the patients earlier and to provide them with the best means to get better chances of cure. They are aware of the fact that several aspects of care are in need of improvement.

They are considered as a model in the country for the multidisciplinary approach and the capacity to build together programs to improve the quality of care. This has to be developed further. Through SMHOP, the group is initiating national programs for treatment. A capacity to identify, to develop adapted program to local conditions and also to monitor the programs is considered as an important tool to develop pediatric oncology in the country. The help of groups like FAPOG and IOP is of great importance in building such capacity. The ongoing experience with UICC is also promising.

No progress can be achieved without social support to patients and parents [9]. The recent interest in cancer in the country, the fact that more patients will have health care insurance are considered as important opportunity to get more support for patients and their families.

There is also a need for the creation of other units throughout the country to take care of children suffering from cancer. Some physicians trained in the pediatric oncology units working in different cities in the Kingdom are able to follow some patients for maintenance therapy or follow up.

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Childhood brain tumours: a developmental disorder

Paul J. Scotting

A major driving force in the study of children's cancers over recent years has been the concept that such tumours are fundamentally different to those in adults. This has led to increased efforts to study these cancers as a unique entity. Here I will focus on issues of children's cancers that arise from studies in the field of developmental biology with specific reference to the brain. I will ask why specific cancers arise in children and why their biology might differ from adult cancers. The answers to these questions relate to the particular behaviour of developing cells, such as their proliferation and plasticity, and the nature of the microenvironment of developing tissues.

Introduction

During development, tissue growth and differentiation is regulated by complex cellular processes that involve precise regulation of both cell division and apoptosis. Defects in any of the pathways that control these processes could promote transformation, making these developing cells particularly prone to tumorigenesis. Tumour formation could therefore be driven by the growth- and differentiation-promoting mechanisms that occur during development. In addition, the immature tissue environment of cells in the developing individual differs from that in adults providing high levels of factors that favour proliferation, migration and plasticity. The pathogenesis of childhood cancer is therefore, intimately linked to the processes of organogenesis, tissue growth and maturation. It is this relationship that seems to explain the unique features that differentiate many childhood cancers from adult-onset tumours.

Organogenesis and tumorigenesis

Proliferation, survival, self-renewal and migration are all aspects of a normal developmental programme. Thus, the cells of most immature tissues already behave in a way that is not dissimilar to a cancer cell. Even with respect to proliferation, a feature that is common to most tumour progenitor cells of adults or children, the dividing cells of developing tissues differ from those in mature tissues. In developing tissues the progeny of most dividing cells are, predominantly, more dividing cells. In addition, these cells often have a high capacity for migratory behaviour and can even be resistant to apoptosis, as epitomized by the rapid expansion of cell numbers in the developing nervous system throughout prenatal development. By contrast, most progeny of dividing cells in mature tissues are fated to initiate rapid differentiation or to undergo relatively rapid apoptosis, such that these processes must be circumvented for a cancer to develop. This type of cellular behaviour is typified by the gut, where most dividing cells rapidly undergo differentiation, although the high level of proliferation makes this a relatively cancer-prone tissue. It is this need to overcome a range of cellular controls that explains the multi-hit process of DNA damage that is required for cancerous development in adults. In children, it seems that fewer cellular features need to change to reach a cancerous state. This led Alfred Knudson to propose that "..a minimum of two events appears to suffice for certain embryonal tumors, leukaemias, and lymphomas for which the target tissues normally show stem cell proliferation". Although there are many children's cancers where the two-hit model is unlikely to apply, a shorter route to tumorigenic transformation than in adult-onset cancers does seem to be a general feature.

Tissue-maturation arrest

Many children's cancers are referred to as embryonal tumours because they originate from immature tissue and their microscopic morphological appearance resembles that of

tissues in the developing embryo and fetus. Post-genomic strategies such as microarray analysis have allowed formal demonstration of the long-standing theory that the biology of cancer cells largely recapitulates the behaviour of cells that are found in developing tissues. For example, gene-expression profiles of the paediatric brain tumour medulloblastoma exhibit a close relationship to the immature cells of the proliferating precursors of the cerebellum.

These observations have led to the model that most CSTs result from defects in mechanisms that control normal development, which arrest the normal pathways of maturation. So, features such as proliferation, plasticity and invasiveness might simply reflect the failure of these cells to progress beyond a stage of development at which these features are normal, rather than being the direct results of the oncogenic transformation of the cell.

The relationship between CST development and organogenesis is supported by the fact that the age-specific pattern of CST incidence reflects the periods of maximum growth of the related normal tissue. This is particularly evident in brain tumours. Medulloblastomas are the most frequent postnatal brain tumour developing in the cerebellum, the main site of postnatal proliferation in the brain. However, although fetal brain tumours are quite rare, these tumours are predominantly teratocarcinomas, reflecting the more primitive stem cells that are present in early brain tissue. Many features of CSTs therefore closely resemble the biology of the immature tissue in which they arise.

Tissue microenvironment

The fidelity of embryonic and fetal development is the end result of a sophisticated interplay between the genetic/molecular machinery of individual cells and the tissue environment in which those cells find themselves. A tissue that contains many rapidly dividing cells not only provides a favourable context for errors in DNA replication but also represents a microenvironment in which proliferation is favoured over differentiation. In addition, the tissue environment of developing cells is one in which the plastic behaviour of early stem cells and proliferating progenitors is kept under tight control, such that the ability of these cells to undergo high levels of proliferation and cell migration is maintained, but such events only occur when they are required for the developmental process.

This influence of the tissue microenvironment on tumour progression is exquisitely illustrated in teratocarcinomas. Tumours of this class, and the embryonic carcinomas that derive from them, have the broadest developmental potential of any tumour type. Such tumours are characterized by the disorganized development of tissues from all three germ layers, producing tissues as diverse as neurons, skin, bone, muscle and endodermal cysts. This broad range of cell types indicates a very primitive stem-cell origin. Although tumours of this type do arise during fetal development in a range of tissue locations, such as brain and heart, they can be experimentally initiated without oncogenic transformation. Transformation can, instead, occur when embryonic stem cells, such as those isolated from the very early mouse embryo, are transplanted into an adult mouse (either subcutaneously or in kidney capsule experiments) — the cells adopt the disorganized tissue differentiation that typifies the teratocarcinoma[1,2]. These tumours can be serially transplanted from adult host to adult host, demonstrating the presence of tumour stem cells[1,2]. Immortal embryo carcinoma (EC) cell lines that are capable of regenerating a teratocarcinoma *in vivo* can be derived from these tumours[1,2]. However, when a teratocarcinoma cell, or even an EC cell that has undergone serial passage in culture, is transplanted back into an embryo, it can readily contribute to normal developmental processes[1,2]. It therefore seems that the normal or malignant behaviour of these cells is entirely dependent on their tissue environment. Presumably, teratocarcinomas that arise during human fetal development are due to these primitive stem cells failing to mature properly before their environment changes to one that cannot instruct their normal differentiation (perhaps due to some genetic error). Or, they could arise due to their aberrant location in a region where those signals are absent (the veracity of these routes has yet to be established).

It is also likely that the biological and clinical

behaviour of other CSTs, in contrast to adult-onset cancers, are also affected by the changing microenvironment of the growing host. This principle might explain spontaneous tumour involution (the process by which some tumours cease to proliferate aggressively and gradually reduce in size) that is seen among CSTs such as capillary hemangioma and infantile stage IVs neurobalstoma.

These various phenomena indicate that many CSTs seem quite sensitive to extracellular signals, and that the appropriate intracellular pathways necessary for the responses to these signals are not disrupted. This responsiveness might reflect their 'mildly transformed' state and lends hope to the potential for targeted therapies that could mimic extracellular differentiating signals.

The mechanism of CST aetiology

Cancers in adults result from a 'multistep' process and often progress over many years or decades. It is striking that children's tumours develop over a much briefer time course, indicating that they might require fewer events to progress and that the mechanisms underlying their initiation might therefore differ from adult cancers.

Despite some reports of ethnic and geographical variation, most types of CST occur with similar worldwide prevalence. One interpretation of this observation is that they are not likely to be caused by environmental factors. Indeed, Knudson stated in 1975 that "The even distribution of certain childhood cancers throughout the world suggests that their incidences are determined by spontaneous mutation rates rather than by local environmental mutagenic carcinogen"[3]. Thus, the defects in cell proliferation and differentiation that lead to CSTs may arise from the physiological imprecision of DNA replication during developmental processes. During normal cell division, despite the activity of mismatch-repair machinery, errors in DNA replication result in the inheritance of 1 mutation in every 10^7 cell divisions, for any given gene[4]. In developing tissues, cells can undergo as many as 10^{10} divisions — a frequency that is certainly sufficient to explain the occurrence of CSTs. These errors of development, and the cancers they cause, might therefore be unpreventable, as their ultimate aetiology could simply be related to the imperfect nature of cell division during the developmental process.

In support of this model, p53 mutations have been reported in CSTs much less frequently[5-7] than in adult-onset cancers[8]. The p53 protein can induce cells to undergo cell-cycle arrest or apoptosis in response to DNA damage. Loss-of-function mutations in p53, which have been reported in a large percentage of adult cancers, allows them to survive in spite of DNA damage and leaves them vunerable to genomic instability[9]. Although some CSTs are associated with inherited mutations in p53[10], the low rate of p53 mutation that is found in most CSTs indicates that these tumours arise not through events that induce DNA damage, but rather through the introduction of mutations that occur as a normal part of the replicative process.

Certain adult cancers (about 15% of colorectal cancers and hereditary Xeroderma pigmentosum) are also driven by DNA-replication errors. However, this route towards oncogenic transformation does not lead to chromosomal instability and aneuploidy, or to the amplification of genetic damage during tumour progression that might be expected (rather, the frequency of point mutations and repeat-sequence slippage are increased)[11]. If DNA-replication error is the main mechanism of CST formation, the prediction would be that CST cells would differ less from normal cells than most adult tumour cells, in which chromosomal instability is an early event in the aetiology of the tumour. This supposition is supported by the anecdotal observation that CSTs generally have fewer cytogenetic defects than adult tumours. These observations lead to a model for the development of CSTs that does not allow them to accumulate genetic damage at the same rate as adult cancers and therefore might explain their comparative curability[7].

An alternative mechanism by which CSTs might arise so rapidly without sequential events of DNA damage, is via epigenetic alterations. Indeed, hypomethylation is a well described early event in many types of cancer. Loss of normal epigenetic features can not only cause deregulation of genes but also genome instability. So is this a mechanism likely to play

a role in CSTs? Epigenetic alterations are used to stabilize gene expression changes established during embryonic development. In addition imprinting of genes by methylation is also lost and then reestablished during embryogenesis. Thus, the epigenetic status of the genome is less defined and more dynamic in the growing individual than in the adult. There is also some evidence that stem cell populations retain an epigenetic state that is more flexible than their more committed counterparts. Thus, such cells could be particularly prone to aberrant alterations that could predispose to cancer.

Summary

CSTs represent a unique group of neoplasias that seem to differ fundamentally from adult-onset cancers, both in their cell biology and their tissue environment. It is therefore likely that effective treatments for these cancers will only be possible when the molecular events that are specific to CST tumorigenesis are better understood. Traditional treatments for cancer have generally targeted the high proliferation rate of tumour cells. This strategy is effective because adult tumours develop in the context of low proliferation in the normal tissue environment. Normal proliferating cells can either be replenished naturally or therapeutically. One challenge of treating CSTs, particularly those that occur in the CNS, is that these tumours are located within a context of rapidly growing normal tissue. Radiation and chemotherapy are particularly hazardous in children as they damage the normal developing organs and cause other tumorigenic events in rapidly dividing normal cell populations. These cancers are therefore in particular need of innovative and targeted therapeutic approaches.

Although CSTs are generally fewer steps away from their normal biology than adult cancers, there are good examples of CST cells that acquire numerous genetic/cellular defects such as higher risk neuroblastomas. An important goal is therefore to develop objective assays that distinguish these two subtypes, since tumour cells with fewer aberrations should respond more readily to biologically targeted intervention. The most attractive strategy for intervention would then be to use agents that can induce either differentiation or apoptosis of these cells. Some such agents are already in the clinic, such as retinoic acid, which mimics the endogenous differentiation signals for sympathoadrenal neurons in the treatment of neuroblastoma, and also induces differentiation of certain leukaemias[12,13].

To fully understand CST cells it will be necessary to compare their molecular features to that of their normal progenitors. This will be a challenge, as many CSTs are composed largely of cells at different stages of development derived from a more rare, malignant, self-renewing tumour stem cell. The main goal will therefore be to identify and analyze both the tumour stem cell and its normal counterpart.

Advances in the isolation and characterization of both normal organ and CST stem cells provide optimism that the events underlying the aberrant behaviour of CSTs may soon be identified. Therapies that target specific alterations in these defective developmental programmes might lead to a significant improvement in the outcome for children who are suffering from these cancers. If CSTs have indeed strayed only a short distance from normal behaviour, it seems reasonable to hope that they will remain sensitive to external factors where adult cancer cells are now beyond such regulation.

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Understanding cellular pathways of DNA damage recognition and repair – clinical implications

Stefan Meyer

The primary molecular structure that carries genomic information - DNA - is constantly subject to alteration and damage by endogenous substrates and environmental factors. Damage can affect DNA in various ways, for example chemical alterations of single bases, single mismatched nucleotides, and more complex alterations such as breaks to single and double strands of DNA molecules, potentially resulting in aneuploidy, deletions, fusions or translocations. For the normal development of the individual cell and the organism as a whole, complex machinery is in place for maintaining the integrity of DNA and the genomic information carried by it to ensure normal development of the organism and prevent malignant transformation and death. The cellular pathways that are in place to recognise defects in DNA structure, and to initiate and carry out the appropriate response, which normally result in either reinstatement of the genomic integrity or cell death, are the subject of current and exciting molecular biological investigations. Damage can be processed by nucleotide excision, base excision, non-homologous or homology directed repair pathways, which all require complex protein/DNA and protein/protein interaction. Although the understanding of these pathways is still incomplete, a picture emerges that shows a cellular network of these pathways closely interacting with each other and other cellular functions involved in cell division, proliferation and differentiation. Proteins that form this network are encoded by more than 150 genes, which facilitate maintenance of DNA integrity. All of these share an interesting biology and here I will attempt to focus on aspects of this important field that are likely to be relevant for the care of children with malignant disease and their families.

Malignant diseases are common and serious complications for individuals with an inherited disruption in genes encoding for proteins involved in DNA damage response pathways. Examples are Ataxia telangiectasia (AT), Bloom syndrome (BS), Nijmegen breakage syndrome (NBS) and Fanconi anaemia (FA). AT is caused by mutations in the *ATM* gene, which functions as a kinase that is activated by ionising radiation and results in microcephaly, progressive cerebral degeneration and immunodeficiency. BS is caused by autosomal recessive inherited mutations in the *BLM* gene, which encodes for a helicase and results in short stature, UV sensitivity and immunodeficiency. NBS is an autosomal recessive disease arising from disruption of the *NBS1* gene, which encodes for the double strand break repair protein *nibrin* that interacts with other essential double strand repair proteins such as MRE11 and RAD51. Clinically NBS shares the phenotypic feature of severe immunodeficiency with AT. Fanconi anaemia is an autosomal or X-linked inherited disease caused by mutations in at least 12 genes, which interact in a common cellular pathway. The Fanconi genes *FANCA, FANCB, FANCC, FANCG, FANCF, FANCM* and *FANCL* encode for proteins, which form a core complex that facilitates the ubiquitination of the FANCD2 protein. Ubiquitinated FANCD2 co-localises with BRCA1 at the site of DNA damage. Downstream in this pathway operate FANCJ and FANCD1/BRCA2. On a cellular level there are important functional interactions between ATM, *nibrin*, BLM and the Fanconi proteins. ATM phosphorylates *nibrin* and FANCD2, while the FA core proteins form complexes with the BLM helicase. The detection of co-localisations signals of these proteins at the site of DNA damage suggests additional direct and indirect interactions. Despite the complex cellular interactions of these proteins and the overlapping clinical features of these disorders, the spectrum of malignancies in each is quite distinct. While AT patients are at

extremely high risk for development of lymphoid malignancies including Hodgkin's disease, T-and B cell Lymphomas and T-cell ALL; NBS patients, in addition to the high risk of lymphomas, also develop brain tumors and rhabdomyosarcomas, which intriguingly appear to arise preferentially peri-anally. Bloom syndrome carries a very high risk of a broad range of malignancies including leukaemias, embryonal tumours and epithelial cancer. FA patients carry an extreme high risk of developing acute myeloid leukaemia (AML) and squamous cell carcinoma, but the spectrum of malignancies in the rare FA-D1 group with bi-allelic mutations in the *BRCA2* gene includes brain tumours, mainly medulloblastoma, and Wilms' tumour as well as T-cell ALL. These patients are also at extreme risk of very early AML, normally presenting within the first three years of life. It is important to consider these genetic conditions in cases that appear to be sporadic malignancies, since patients with NBS or FA can have a very subtle phenotype but still experience extreme toxicity when treated with chemotherapy or radiation, as these pathways play an important role in the cellular response to chemotherapy and radiation. Several studies and reports have suggested that inherited chromosomal fragility syndromes are frequently overlooked or unrecognised. The detection of *BRCA2* as the Fanconi gene mutated in the rare FA-D1 group also implies that the correct diagnosis can have important implications for the parents and siblings in terms of cancer risk in the future, which might not be evident from a superficially taken family history.

Due to the overall rarity of these conditions the question as to whether malignancies arising from chromosomal instability syndromes have the same biology as sporadic childhood malignancies is difficult to answer. On the other hand, erroneous repair of DNA breaks has been implicated in the origin of chromosomal translocations that are characteristic for many childhood malignancies including leukaemia, lymphomas and solid malignancies. This implies the possibility that the same mechanisms that cause malignant transformation in genetic defects of DNA damage recognition and repair may be involved in sporadic childhood cancers. Little is known at present about the dynamics of the DNA damage recognition and repair machinery during prenatal development, when many childhood malignancies appear to be initiated. Whether this follows the same dynamics as in the postnatal period will be an interesting question to address in the future. Detailed cytogenetic investigations of myelodysplastic syndrome (MDS) and AML in FA patients, however, point to genetic events on chromosome 3q that are strongly associated with malignant transformation and progression in haematological malignancies arising from an inherited defect in the FA pathway, which does not appear to involve a common non-random chromosomal translocation characteristic for childhood leukaemia. Together with the observation that FA-derived AML in general lacks typical chromosomal translocations characteristic of childhood leukaemia, implies the possibility that the biology of malignancies arising from an inherited disruption in DNA damage recognition and repair might be different from that of sporadic childhood malignancies. Characterising the genetic changes also of other malignancies in patients with inherited defects will give further answers to these important questions.

Several studies have addressed the role of genetic variations and heterozygous carrier status in genes involved in DNA damage recognition and repair for childhood malignancies. These have included *ATM*, *NBS1* and some of the FA genes as well as other DNA damage recognition and repair genes and concentrated mainly on leukaemia and lymphomas. Although several genetic variants have been identified that might confer an increased risk for certain malignancies, all of these studies were limited by relatively small patient numbers, which in general limits the value of genetic epidemiology for paediatric oncology. In adults, however, heterozygous carrier status of mutations in *ATM*, *NBS1, BLM* and certain FA-genes – especially *FANCD1*/*BRCA2*, - appear to confer an increased cancer risk, which includes haematological malignancies as well as epithelial cancer. Intriguingly the spectrum of malignancies can be very different as exemplified by one of the clinically most important cancer genes, *BRCA2*: While bi-allelic mutations in *BRCA2* are

associated with medulloblastoma, Wilms' Tumour and very early AML, heterozygous carrier status of *BRCA2* mutations gives raise to breast, ovarian and prostate cancer. This implies additional roles of the BRCA2 protein in early development that so far had little attention paid to it.

Many childhood malignancies as well as adult cancers share features of chromosomal instability and chemo-sensitivity with cells carrying a constitutional disruption in a DNA damage recognition and repair function, such as FA or NBS. This implies the possibility that in sporadic malignancies these pathways might be somatically disrupted. Somatic alteration and disruption of the *ATM* gene has been demonstrated in a number of sporadic lymphoid malignancies, mainly in adults, but also in children and implies a role of acquired ATM dysfunction in a proportion of sporadic lymphoid malignancies. Methylation induced silencing of the *FANCF* gene resulting in a acquired dysfunction of the FA pathway has been suggested to play an important role in some adult type epithelial cancers and to confer an important mechanism of platinum sensitivity. However, other studies have not detected methylation of *FANCF* and other FA genes in ovarian tumours and childhood leukaemia. There are, however, numerous other mechanisms that could result in somatic disruption of DNA damage response pathways and confer chromosomal instability and chemo sensitivity. These include other forms of modulation of gene expression or posttranslational modifications. Investigations addressing somatic disruption and acquired dysfunction in DNA damage recognition and repair pathways in malignant cells could prove very important in the future. Targeting the inherited DNA repair defect in *BRCA2*-associated breast cancer appears to be an important novel therapeutic strategy that can be pharmacologically exploited. Inhibition of the base excision repair protein Poly(ADP-ribose)polymerase (PARP) in BRCA2 deficient cells with experimental compounds has been shown to result in profound chemo-sensitisation and is currently the subject of pilot clinical investigations. These strategies might not only be important in cancers arising from a constitutional defect in DNA repair, where they could influence treatment of children with malignancies arising from a constitutional defect in DNA damage recognition and repair. If these pathways prove to be somatically mutated in childhood malignancies, an approach that targets an acquired defect in DNA damage response pathways might increase the effects of chemotherapy without resulting in increased toxicity.

In summary, understanding DNA damage recognition and response pathways might not only help to manage rare forms of childhood cancer in children with a constitutional defect in these pathways, but also contribute to the understanding why and how childhood cancer is initiated and progresses. In addition, as these pathways are involved in the cellular response to chemotherapy, understanding these pathways might result in novel therapeutic strategies.

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Pathogenic Mechanisms in Juvenile Myelomonocytic Leukemia

Kevin Shannon

Juvenile myelomonocytic leukemia (JMML) is a relentless myeloproliferative disorder (MPD) of young children characterized by over-production of myeloid lineage cells that infiltrate hematopoietic and non-hematopoietic tissues including skin, lung, and spleen. The median survival is < 1 year without hematopoietic stem cell transplantation (HSCT). A cellular hallmark of JMML is that primary blood or bone marrow cells form abnormal numbers of colony forming unit granulocyte-macrophage (CFU-GM) colonies in methylcellulose cultures containing low concentrations of the growth factor granulocyte-macrophage colony-stimulating factor (GM-CSF). Children with neurofibromatosis type 1 (NF1) and Noonan syndrome (NS) are at increased risk of developing JMML and studies of these inherited cancer predispositions implicate hyperactive Ras signaling as an initiating event in leukemogenesis.

Ras proteins regulate cell fates by cycling between an active guanosine triphosphate (GTP)-bound and an inactive guanosine diphosphate (GDP)-bound conformations (Ras-GTP and Ras-GDP). Ras-GTP interacts productively with downstream effectors including phosphoinositide-3-OH (PI3) kinase, Raf1, and Ral-GDS to regulate multiple cellular processes including proliferation, survival, and differentiation. The intrinsic Ras GTPase terminates signaling by hydrolyzing Ras-GTP to Ras-GDP. This slow "off" reaction is greatly augmented by GTPase activating proteins (GAPs), which bind to the effector domain of Ras-GTP and accelerate its conversion to Ras-GDP by stabilizing a transition state between Ras-GTP and Ras-GDP. Somatic point mutations in the *NRAS* and *KRAS2* genes occur in myeloid malignancies and many other human cancers, including about 25% of JMML cases. These mutations introduce amino acid substitutions at codons 12, 13, and 61 and encode proteins that accumulate in the GTP-bound conformation due to defective intrinsic GTPase activity and resistance to GAPs. Neurofibromin, the *NF1* gene product, is a GAP for Ras.

Children (but not adults) with NF1 are strongly predisposed to JMML and other myeloid malignancies. The dominantly-inherited cancer predisposition in persons with NF1, the frequent occurrence of *NRAS* and *KRAS2* in human malignancies, and the identification of neurofibromin as a GAP for Ras suggested that *NF1* might function as a tumor suppressor gene (TSG) in JMML. Consistent with the model, JMML bone marrows from children with NF1 frequently show loss of constitutional heterozygosity (LOH) at the *NF1* locus, which invariably involved deletion of the normal parental allele in familial cases. A study that demonstrated homozygous inactivation of *NF1* in JMML bone marrows provided formal genetic proof that the *NF1* gene functions as a TSG. In addition, JMML samples from children with NF1 showed a reduction of neurofibromin GAP activity, elevated levels of Ras-GTP, and activation of the Raf1 effector ERK. Together these data identified *NF1* as a myeloid TSG that functions by negatively regulating Ras signaling.

NS is a dominant developmental disorder characterized by cardiac defects, facial dysmorphism, and skeletal malformations. Clinical reports described a spectrum of hematologic abnormalities including isolated monocytosis, a, CMML-like disorder that remits spontaneously, and JMML. As in NF1, the discovery of germline missense mutations in the *PTPN11* gene as the underlying cause of ~50% of NS cases by Marco Tartaglia, Bruce Gelb and their collegues proved crucial for unraveling the mechanism of aberrant myeloid proliferation in NS patients and unexpectedly identified *PTPN11*

as the most common target of somatic mutations in JMML. *PTPN11* encodes SHP-2, a cytosolic non-receptor tyrosine phosphatase that includes two Src homology 2 (SH2) domains (termed N-SH2 and C-SH2) and a catalytic protein tyrosine phosphatase (PTP) domain. The SHP-2 N-SH2 domain inhibits phosphatase activity by blocking the active site of the PTP domain. The SHP-2 PTPase becomes activated when its SH-2 domains bind an appropriate tyrosine phosphorylated ligand, which induces a conformational shift that "opens" the active site. In contrast to many other PTPases, SHP-2 generally plays a positive role in transducing signals from these receptors, which is mediated, at least in part, through Ras-GTP. SHP-2 is expressed at high levels in hematopoietic cells and undergoes rapid tyrosine phosphorylation upon activation of the c-kit, interleukin 3 (IL-3), GM-CSF, and erythropoietin receptors.

Most of the germline *PTPN11* mutations found in NS constitutively activate SHP-2 phosphatase activity by destabilizing auto-inhibitory interactions between the N-SH2 and PTP domains of the protein. The association between NS and JMML and the key roles of SHP-2 in Ras signaling and hematopoiesis suggested that *PTPN11* might be mutated in patients with JMML. Indeed, this hypothesis was confirmed by studies that identified novel heterozygous germline *PTPN11* mutations in patients with NS and JMML. Importantly, somatic mutations in *PTPN11* occur in approximately 35% of all patients with JMML. *PTPN11*, *KRAS2, NRAS*, and *NF1* mutations are rarely detected in the same patient, which is consistent with the fact that these genes encode components of the same growth control pathway. Although all of the somatic *PTPN11* mutations identified in JMML are missense changes, both the spectrum and distribution of these mutations differs from NS. These leukemia-associated mutations encode stronger gain-of-function mutations than those found in NS and it is therefore likely that they would not be tolerated in the germline. The less severe biochemical phenotype of the mutations found in children with NS likely explains clinical observation that many of the hematological abnormalities detected in infants with NS resolve without soecific treatment. Most recently, a child with NS and JMML was identified with a novel germline *KRAS2* mutation that deregulates the growth of primary hematopoietic progenitors and induces elevated levels of phosphorylated MEK and Akt (p MEK and pAkt) in cultured macrophages.

Genetically engineered strains of mutant mice have been extremely valuable for analyzing the functional consequences of genes that are mutated in JMML. Although homozygous inactivation of *Nf1* is lethal in embryonic life, fetal hematopoietic cells demonstrate a similar pattern of hypersensitive CFU-GM colony growth in response to GM-CSF as human JMMLs and adoptive transfer into irradiated recipients induces a JMML-like MPD with hyperactive Ras. Studies in this mouse model also showed that *Nf1* inactivation leads to deregulated growth in multiple hematopoietic compartments and confers a durable proliferative advantage in competitive repopulation assays. A cross between *Nf1* and *Gmcsf* mutant mice also showed that aberrant GM-CSF signaling plays a central role in initiating and maintaining the JMML-like MPD. The JMML model that was developed by injecting *Nf1*-deficient fetal liver cells into irradiated recipient mice was also used to perform a preclinical trial that evaluated the efficacy of an inhibitor of the Ras processing enzyme farnesyltransferase, which included pharmacodynamic monitoring in primary hematopoietic cells. This study showed no inhibition of K-Ras and N-Ras processing at the maximal tolerated dose, and no improvement in the MPD. A conditional mutant allele of *Nf1* was generated to overcome the embryonic lethality that results from homozygous *Nf1* inactivation. Somatic inactivation of this $Nf1^{flox}$ allele in hematopoietic cells and found that that this consistently results in a JMML-like MPD that is associated with leukocytosis, splenomegaly, hyperproliferation, impaired apoptosis, and *in vitro* hypersensitivity to GM-CSF. Using the same strategy to activate a conditional "knock-in" allele to induce expression of an oncogenic $K\text{-}Ras^{G12D}$ protein from the endogenous locus by excising a loxP-stop-loxP (LSL) cassette. Compound *Mx1-Cre* $LSL\text{-}Kras^{G12D}$ mutant mice also develop an aggressive MPD with leukocytosis and death at a median 105 days of age. Bone marrow cells and splenocytes demonstrate profound hypersensitivity in response to GM-CSF and form

many large CFU-GM in methylcellulose cultures containing low concentrations of GM-CSF and other myeloid growth factors. Finally, a mouse model of NS colleagues was generated by constitutively expressing an amino acid substitution (D61G) identified in human patients from the endogenous murine *Ptpn11* locus. Heterozygous *Ptpn11*^{D61G} mutant mice showed cardiac and skeletal defects and develop a subacute MPD that models some aspects of JMML. Interestingly, D61G is the only *PTPN11* mutation that is detected in NS patients with and without leukemia as well as in sporadic JMML.

Together, the germline and somatic mutations found in children with JMML and evidence from strains of *Kras, Nf1,* and *Ptpn11* mutant mice strongly support the hypothesis that genetic lesions that result in hyperactive Ras play a central role in the pathogenesis of JMML. Understanding the association of JMML with NF1 and NS has helped to uncover genes and proteins that are critical for normal growth control and, when deregulated, contribute to leukemia. The studies performed to raise a number of biologic and clinical questions. An intriguing question is why the risk of leukemia in persons with NF1 and NS is confined to a narrow developmental window. Children with these diseases who do not develop a myeloid malignancy during the first few years of life do not appear to be at increased risk of developing hematologic malignancies later in life despite the fact that the bone marrow remains highly proliferative. This clinical observation, in turn, suggests something unique about fetal hematopoietic stem cells and/or the fetal microenvironment that increases the probability that a clone that has sustained a mutation that deregulates Ras signaling will persist and ultimately cause JMML. Finally, genetic and biochemical data have identified hyperactive Ras as a rational biochemical target for treating children with JMML. Unfortunately, the inhibiting aberrant signaling at the level of Ras itself requires restoring enzymatic activity (i.e. accelerating the intrinsic GTPase), which is an exceedingly difficult phamacologic problem. For this reason, efforts have focused on discovering small molecules that inhibit components of effector kinase cascades that are activated downstream of Ras-GTP. *Kras* and *Nf1* mutant mice and cells from these animals provide robust reagents for testing novel therapeutic strategies that may ultimately improve the treatment of children with JMML.

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Minimally Invasive Diagnosis and Treatment of Pediatric Malignancies through Interventional Radiology

Fredric A. Hoffer

Minimally Invasive Diagnosis

Percutaneous biopsy of solid tumor malignancies is common in adult medicine and has not caught on in centers outside of major pediatric oncology practices. Percutaneous biopsy of Ewing sarcoma family of tumors (ESFT) [1], neuroblastoma [2] and chest lesions [3] was quite successful at the Children's Hospital in Boston and successfully obtained diagnostic and prognostic information from cytogenetics [4] including the t(11;22) of ESFT, fluorescent in site hybridization techniques for neuroblastoma *MYCN* amplification and flow cytometry for DNA index. Percutaneous biopsy of solid tumors was the subject of a fine paper from Great Ormond Street Hospital for Children at last years SIOP meeting [5]. At St. Jude Children's Research Hospital, the interventional radiologist does more tumor biopsies than any surgeon [6]. Molecular pathology and immunohistochemistry have advanced the diagnostic powers of imaging guided needle biopsies. Many pediatric centers are slow to catch on due to inadequate tissue by non-aggressive radiologists or due to reluctant pathologists and aggressive surgeons. Many core specimens from 15 or 16 gauge needles obtain sufficient material with minimal risk. Shimada [7] has required incisional biopsies for his neuroblastoma classification as favorable or unfavorable. However, the MK index can be determined from a needle specimen and the gross pathology from diagnostic imaging. Hopefully the new International Neuroblastoma Staging System presented at this meeting will not require open biopsy.

Percutaneous biopsy of the lung and transjugular biopsy liver [8, 9] have offered relatively safe methods to diagnose infections and other complications of cancer treatment.

Fluid aspiration of hematologic malignancies of non-Hodgkin lymphoma (NHL) and leukemia is the next logical diagnostic step after bone marrow aspiration [10]. Flow cytometry can characterize the B or T cell type of the blasts from this fluid. If this step fails to be diagnostic, core needle biopsy will most often succeed and often catches the Reed Sternberg cell needed to diagnose Hodgkin lymphoma or immunohistochemistry for the NHL.

Fine needle aspiration (FNA) of thyroid nodules after radiotherapy may be able to differentiate the malignant papillary carcinoma but may be unable to differentiate normal follicular cells from an adenoma or carcinoma. Thyroid lesions that are over 1 cm in size or that are symptomatic may benefit from FNA [11].

Needle localization of small tumors for surgical diagnostic or therapeutic resection [12] may be necessary for musculoskeletal lesions, lymph nodes or lung nodules especially if the minimally invasive thoracoscopic techniques are used. However, ultrasound guided biopsy of pleural based nodules [13] has been successful in lesions as small as 2 mm. Perhaps the interventional radiologist can simply ablate these lesions in the future.

Minimally invasive treatment

Thermal ablation of pediatric malignancies using radiofrequency ablation (RFA) is in a Phase I study at St. Jude Children's Research Hospital [14-16]. This technology is widely used in adults for hepatocellular carcinoma or colon metastases in the liver. I have successfully ablated rhabdomyosarcoma, adenocarcinoma of the colon, pancreatoblastoma and fibrolamellar hepatocellular carcinoma of the liver (unpublished) but only the last patient remains alive. It is largely a palliative treatment but may lengthen the patient's life with limited toxicity. The liver regenerates. Large lesions may benefit from chemoembolization prior to RFA.

Lung RFA is becoming more common in adult patients than liver RFA treating both lung metastases and primary adenocarcinoma of the lung. I have treated 12 patients with pediatric acquired disease (unpublished) metastatic to the lung. The inclusion criteria include a prior thoracotomy. Since 90% of osteosarcomas recur in the lung after a thoracotomy and 50% recur in the scar, there is room for improvement [17]. I have treated mainly patients with osteosarcoma including one pediatric patient with ablated pulmonary metastases who is alive and well at 25 months post first RFA. Other pulmonary metastases ablated include Wilms tumor, synovial sarcoma, hepatoma and adrenocortical carcinoma. Four are alive with disease at a median of 17 months and seven are dead of disease at a median of 11 months after the first RF ablation. One of these last patients went to Michigan for cryoablation which was much less painful than RF ablation.

Musculoskeletal RFA has been very helpful in adults with painful metastases and is competing with radiotherapy for palliation because the pain relief is immediate. I have performed RFA on five pediatric patients with good success in truncal lesions (rhabdomyosarcoma of breast, leiomyosarcoma of ribs). However the head/neck and extremity lesions are far away from the grounding pads and the high impedance inhibits effective ablation. Perhaps bipolar RF, cryotherapy, microwave, or high intensity focused ultrasound (HIFU) will be more useful in these lesions since they do not require ground pads.

Both cryotherapy and RF ablation have been performed in adults with peripheral renal cell carcinoma [18]. I am hoping that thermal ablation can be used to treat malignant lesions after nephron sparing surgery of nephroblastomatosis and Wilms tumor [19].

Thrombolysis and venous angioplasty is useful to restore central venous patency and may be required in pediatric oncology patients who are hypercoagulable from chemotherapy or who have had subclavian venous access. Central venous line placement by imaging guided internal jugular access has the least thrombosis/stenosis rate [20].

Pleurodesis for malignant pleural effusions at the end of life may allow patients to return home without a chest tube [unpublished]. After percutaneous chest tube placement, Doxycycline can be placed in and within 30 minutes removed from the pleural space under general anesthesia with sclerosis of the pleura occurring within 24 hours.

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Osteosarcoma

Stefan S. Bielack

Abstract

Osteosarcoma is the most frequent primary cancer of bone. When treated by surgery alone, it is almost invariably followed by metastatic dissemination and death. This dismal prognosis can be improved dramatically by including chemotherapy in an interdisciplinary regimen. Today, some two thirds of patients with localized extremity primaries can achieve long-term survival with such intensive multimodal therapy. This article provides a summary overview of current pharmacotherapy in osteosarcoma of the extremities, focussing on the approach of preoperative "neoadjuvant" chemotherapy and thus the potential benefits and pitfalls of delaying surgery. Prospective, multi-institutional trials are essential in guaranteeing that as many patients as possible can benefit from modern, efficacious interdisciplinary therapeutic regimens and that further progress can be made.

Introduction

Osteosarcoma is the most frequent primary solid malignancy of bone. It is defined by the presence of malignant mesenchymal cells which produce osteoid or immature bone[1,2]. This manuscript will focus on high-grade central osteosarcoma, the most frequent subtype[1,3], while readers interested in rarer variants such as parosteal [4,5], periosteal [6,7], high-grade surface [8] or low-grade central osteosarcomas [9-11] or osteosarcomas of the craniofacial bones [12] are asked to refer to the literature.

The incidence of osteosarcoma in the general population is only 2-3 per million per year. It is much higher in adolescents, where the annual incidence peaks at 8-11 per million at age 15-19 years and the tumor accounts for more than 10% of all solid malignancies. Males are affected approximately 1.4 times more often than females [13,14]. Osteosarcoma is usually located in the metaphysis of a long extremity bone, most commonly the distal femur, the proximal tibia, or the proximal humerus. The axial skeleton or craniofacial bones are primarily affected in older patients [1,2]. While several exogenous risk factors, such as radiation or exposure to alkylating agents [15-17], and several well defined genetic predispositions, most noteably hereditary retinoblastoma, the Li-Fraumeni-cancer family syndrome, and Rothmund-Thomson syndrome are associated with an increased risk of osteosarcoma [3], the vast majority apparently arise spontaneously.

High-grade osteosarcomas have a great propensity to metastasize. Primary as well as metachronous metastases usually involve the lungs or, less frequently, distant bones, while other sites are only rarely affected [1,2,18,19]. At diagnosis, even the most accurate staging procedures detect metastases in only 10-20% of patients, but without adequate treatment, most patients with seemingly localized disease will develop secondary metastases and die within one to two years[1,2,18-20]. Today, approximately 50-70% of patients can hope to achieve long-term survival with an interdisciplinary treatment including surgery and multidrug chemotherapy.[21] Inappropriate use of diagnostic tools and suboptimal therapy, however, can still irrevocably compromise a patient's chance for cure. Therefore, affected patients should be treated in specialized centers able to provide access to the full spectrum of care. In many countries, it is standard clinical practice to provide such care within the frame of prospective clinical trials. These trials have been essential in guaranteeing that as many patients as possible can benefit from modern, efficacious interdisciplinary therapeutic regimens.

Goal of therapy

Local treatment of osteosarcoma remains a domain of surgery, while chemotherapy is

needed to achieve systemic control. The goal of surgery is to savely remove the tumor and yet preserve as much extremity function as possible. Local control is achieved by surgery with resection margins which are at least "wide". According to Enneking a wide resection implies complete removal of the tumor (including the biopsy scar) surrounded by an unviolated cuff of normal tissue[22] **(Table 1)**. Depending on the individual anatomical situation, this can be achieved either by ablative techniques such as amputation or disarticulation, by a rotation-plasty, or by limb-salvage procedures. Permanent local control can be achieved in approximately 95% of patients with extremity osteosarcomas [21,23]. Currently, most patients are offered limb-salvage surgery in which the defect left by an en-bloc resection is reconstructed with an endoprosthesis or biological material such as free or vascularised autografts or allografts[21,24,25]. If metastases are large enough to be radiologically detectable, these must also be operated, as they will otherwise progress and result in death [26]. Radiotherapy has a very limited role in the local therapy of osteosarcoma and should be reserved for inoperable situations.

Table 1: Surgical margins in musculoskeletal oncology [22]

Type	Dissection
Intralesional	within the lesion
Marginal	through the pseudocapsule or reactive tissue
Wide	lesion (including biopsy scar), pseudocapsule and/or reactive zone, and an unviolated cuff of normal tissue completely surrounding the mass removed as a single block
Radical	entire anatomic compartment containing the tumor removed as one block

Despite excellent local control rates with surgery, most patients will die from pulmonary metastases if no additional, systemic therapy is added [1,2,18]. The primary goal of chemotherapy within the multimodal treatment of osteosarcoma therefore is to eradicate micrometastatic disease. In addition, effective chemotherapy may aid to increase the safety of surgery [23,27]. Chemotherapy alone is insufficient to reliably destroy either the primary tumor [28] or to eradicate clinically detectable metastases [26]. Chemotherapy can either be administered after surgery (named postoperative or adjuvant chemotherapy) or before surgery (named preoperative, induction, or neoadjuvant chemotherapy). Most current protocols employ chemotherapy both before and after surgery. Details of chemotherapy scheduling will be discussed elsewhere in this chapter.

Available compounds

The list of drugs which are currently employed against osteosarcoma is rather short. Results of phase II trials in osteosarcoma are notoriously difficult to interpret, as response of bone lesions is hard to assess and lung metastases may fail to shrink even when most or all tumor cells have been destroyed, because they often contain considerable amounts of osteoid matrix. A review of 193 osteosarcoma patients included in Pediatric Oncology Group phase II trials, for instance, came up with an overall response rate of only 10.7%, the lowest of all sarcomas tested [29]. Currently, high-dose methotrexate, doxorubicin, cisplatin, and ifosfamide are considered the most active drugs against osteosarcoma (see below).

Methotrexate, the classical antifolate, blocks the action of dihydrofolate reductase. Together with doxorubicin, it was among the very first agents to show efficacy against osteosarcoma [30,31]. In osteosarcoma, methotrexate is given as high-dose treatment with doses of 8 to 12g/m^2. Toxicity must then be antagonized by the antidote leucovorin, activated folate, bypassing the blocked enzyme. The treatment concept of high-dose methotrexate is based on the assumption that normal cells can be rescued more effectively than tumor cells, which may lack active folate transporters.

High dose methotrexate therapy requires meticulous attention and extensive supportive measures, including hydration, alkalinization of the urine, and leucovorin administration which must be adapted to methotrexate serum levels [32-34]. With such supportive care, high-dose methotrexate is tolerated well by most patients. Some patients, however, will still experience

severe toxicity, the risk apparently increasing with increasing patient age. The development of acute renal failure, mediated by the precipitation of methotrexate and its metabolites in the renal tubules [35], is a potentially life-threatening complication. Methotrexate is primarily cleared by renal excretion [34], and renal dysfunction therefore results in delayed methotrexate clearance and sustained, elevated plasma methotrexate levels. This is associated with a marked enhancement of methotrexate's other toxicities, such as myelosuppression, mucosal, or skin toxicity [32-34]. Approximately 1.8% of osteosarcoma patients receiving high-dose methotrexate develop significant nephrotoxicity and the mortality among these patients is 4.4% [36]. Dialysis-based methods of methotrexate removal have limited effectiveness in removing methotrexate compared to rapid reductions in plasma methotrexate concentrations achieved with carboxypeptidase G2, an enzyme which cleaves methotrexate, which should be considered in selected patients with renal failure and severely delayed methotrexate clearance [36]. Most cases of delayed methotrexate clearance, however, can be successfully managed with high-dose leucovorin as sole therapy [37].

The anthracycline doxorubicin (adriamycin) was first introduced into osteosarcoma treatment in the early 1970s [38-40]. There is almost no recent or current treatment regimen which does not include this agent, usually at individual doses ranging from 60 to 90 mg/m^2 and cumulative doses in the range of 300 to 450 mg/m^2 (**Table 2** [18,41-60]). Anthracycline therapy can be associated with both early and late cardiotoxicity and doxorubicin administration to young patients with a fairly high cure rate is therefore not without risks. Severe or even fatal cardiomyopathy during long-term follow-up of otherwise successfully treated osteosarcoma patients has been reported [51,53].

The efficacy of cisplatin against osteosarcomas was proven in early phase II trials [61,62] and the agent was subsequently incorporated into most multiagent regimens. Cisplatin therapy requires supportive hyperhydration. Oto- and nephrotoxicity are dose limiting toxicities. Both can be reduced by administering the drug as a continuous infusion. Intraarterial cisplatin administration directly into the artery supplying the tumor was investigated in the 1980s, but was later largely abandoned when no enhanced antitumor effects compared to intravenous administration could be demonstrate in a controlled trial [63].

Ifosfamide has proven its activity against osteosarcoma in several phase II trials [64-67] and has been included in a variety of polychemotherapy protocols for osteosarcoma **(Table 2)**. Originally, ifosfamide doses in the range of 5 to 8 g/m^2 were used against osteosarcoma, but higher doses of 12 up to 18 g/m^2 may be more efficacious [65,68,69]. A Pediatric Oncology Group study found responses in 9 of 33 chemotherapy naive and in 3/30 pretreated osteosarcomas at an ifosfamide dose of 12 g/m^2 [65]. The French Society of Pediatric Oncology observed 13 responses in 27 relapsed or refractory osteosarcomas with 12 g/m^2 ifosfamide plus moderate dose etoposide. Their conclusion was, that this combination of drugs has tolerable toxicity, is efficient and would deserve evaluation in phase III studies [68]. A study from North-America adding high-dose ifosfamide and etoposide to a three drug regimen for patients with primary metastatic osteosarcoma reported a response rate of 62% and a 2-year event-free survival of 45.[69]

The administration of ifosfamide requires supportive measures to prevent the otherwise frequent hemorrhagic uropathy, namely hydration and the administration of Mesna (Uromitexan). High-dose ifosfamide therapy may be associated with significant, albeit mostly reversible, central nervous system toxicity. ifosfamide may also lead to chronic renal tubular toxicity, the Fanconi syndrome, and to sterility [70].

No other agent has come close to replacing the four standard substances described above. Carboplatin has demonstrated some activity [71,72], but the results derived from a phase II study in advanced tumors [73] and a Pediatric Oncology Group phase II window trial in newly diagnosed metastatic or unresectable osteosarcoma [72] suggest that it may be less active than cisplatin. Etoposide has shown limited activity when administered as a single agent [74] but may enhance the effect of other drugs such as carboplatin[55], cyclophosphamide [75,76], or

Table 2: Results of selected osteosarcoma protocols for localized extremity osteosarcoma.

Protocol	Patients	Preoperative chemotherapy	Response	Postoperative chemotherapy	Event-free	Reference
T-7	61	MTX-HD, DOX, BCD, VCR	any	MTX-HD, DOX, BCD, VCR	80% at 3 years	ROSEN 1981 [41]
T-10	57	MTX-HD, DOX, BCD	good poor	MTX-HD, DOX, BCD DOX, DDP, BCD	93% at 1.7 years	ROSEN 1982 [42]
COSS-80	116	MTX-HD, DOX, , DDP MTX-HD, DOX, BCD	any any	MTX-HD, DOX, DDP MTX-HD, DOX, BCD	68% at 2.5 years	WINKLER 1984 [43]
MIOS	18 & 59* 18 & 18*	MTX-HD, DOX, DDP, BCD none	any -	MTX-HD, DOX, DDP, BCD none	66% & 69%* at 2 years 17% & 9%* at 2 years	LINK 1986 [18]
COSS-82	59	MTX-HD, DOX, DDP	good poor	MTX-HD, DOX, DDP DDP, IFOS, CYC, ActoD	68% at 5 years	WINKLER 88 [44]
	60	MTX-HD, BCD	good poor	MTX-HD, BCD DOX, DDP	45% at 5 years	
IOR/OS-1	127	MTX-HD, DOX, DDP, BCD MT-ID, DOX, DDP, BCD	any any	MTX-HD, DOX, DDP, BCD* MTX-ID, DOX, DDP, BCD*	58% at 5 years 42% at 5 years	BACCI 1990 [45]
SSG-T10	97	MTX-HD	good poor	MTX-HD, BCD MTX-HD, DOX, DDP, BCD	54% at 5 years	SAETER 1991 [46]
EOI-80831	99 99	DOX, DDP MTX-HD, DOX, DDP	any any		57% at 5 years 41% at 5 years	BRAMWELL 1992 [47]
T4-T12	279	MTX-HD, DOX, DDP, BCD**	any	MTX-HD, DOX, DDP, BCD**	65% at 5 years	MEYERS 1992 [48]
EOI-80861	199	DOX, DDP	any	DOX, DDP	44% at 5 years	SOUHAMI 1997 [49]
	192	MTX-HD, DOX, DDP, IFOS, BCD, VCR	any	MTX-HD, DOX, DDP, IFOS, BCD, VCR	44% at 5 years	
CCG-782	268	MTX, BCD	good poor	MTX-HD, DOX DOX, DDP, BCD	53% at 8 years	PROVISOR 1997 [50]
COSS-86	171	MTX-HD, DOX, DDP +/- IFOS	any	MTX-HD, DOX, DDP +/- IFOS	66% at 10 years	FUCHS 1998 [51]
T-12	37 36	MTX-HD, BCD MTX-HD, DOX, DDP, BCD	good poor any	MTX-HD, DOX, BCD MTX-HD, DOX, DDP, BCD MTX-HD, DOX, DDP, BCD	73% at 5 years 78% at 5 years	MEYERS 1998 [52]
IOR/OS-2	164	MTX-HD, DOX, DDP	good poor	MTX-HD, DOX, DDP MTX-HD, DOX, DDP, IFOS, ETO	59% at 10 years	BACCI 2000 [53]
IOR/OS-4	133	MTX-HD, DOX, DDP, IFOS	any	MTX-HD, DOX, DDP, IFOS	56% at 5 years	BACCI 2001 [54]
OS-91	47	IFOS, CARBO	not PD PD	MTX-HD, DOX, IFOS, CARBO MTX-HD, DOX, DDP	73% at 3 years	MEYER 2001 [55]
IOR-PILOT	68	MTX-HD, DOX, DDP, IFOS	any	MTX-HD, DOX, DDP, IFOS	73% at 4 years	BACCI 2002 [57]
EOI 86294690	250 254	DOX, DDP DOX, DDP + G-CSF	any any	DOX, DDP DOX, DDP + G-CSF	41%at 3 years 46% at 3 years	LEWIS 2003 [58]
POG-8651	45 55	HD-MTX, DOX, DDP, BCD none	any -	HD-MTX, DOX, DDP, BCD HD-MTX, DOX, DDP, BCD	61% at 5 years 65% at 5 years	GOORIN 2003 [59]
SSG-VIII	113	MTX-HD, DOX, DDP	good	MTX-HD, DOX, DDP	63% at 5 years	SMELAND 2003 [60]
INT 0133	172 168 167 170	MTX-HD, DOX, DDP MTX-HD, DOX, DDP MTX-HD, DOX, IFOS MTX-HD, DOX, IFOS	any any any any	MTX-HD, DOX, DDP MTX-HD, DOX, DDP + MTP-PE MTX-HD, DOX, DDP, IFOS MTX-HD, DOX, DDP, IFOS + MTP-PE	71% at 3 years 68% at 3 years 61% at 3 years 78% at 3 years	MEYERS 2005 [56]
ISG/SSG I	182	MTX-HD, DOX, DDP, IFOS-HD	any	MTX-HD, DOX, DDP, IFOS-HD	64% at 5 years	FERRARI 2005 [67a]
BOTG III/IV	168	IFOS, CARBO, EPI IFOS, CARBO, EPI DOX, DDP, CARBO	any*** any*** any	IFOS, CARBO, EPI MTX-HD, IFOS, CARBO, EPI DOX, DDP, IFOS, CARBO	46% at 5 years	PETRILLI 2006 [67b]

Abbreviations: BCD = bleomycin + cyclophosphamide + dactinomycin, CARBO = carboplatin, DDP = cisplatin, DOX = doxorubicin, EPI = epirubicin, ETO = etoposide, G-CSF = granulocyte colony stimulating factor, IFOS = ifosfamide, MTX = methotrexate, VCR = vincristine, HD = high-dose, ID = intermediate dose, PD = progressive disease

** randomized and non-randomized patients* *** Drugs in varying combinations.* **** Patients requiring amputation or presenting with large tumors (>12 cm) received regimen B*

ifosfamide [68,69]. 5-azacytidine[77], Daunorubicin [78], 5-Fluorouracil/Leucovorin [79], paclitaxel [80,81], docetaxel [82,83], iproplatin [84,85], Ecteinascidin-743 [86], and topotecan [87] seem to be more or less inactive when given as single agents, while gemcitabine has shown marginal activity [88]. The results achieved with high-dose chemotherapy and autologous peripheral blood stem cell rescue have not been promising in the few reported series [89-91].

Osteosarcoma is unique among tumors as it produces osteoid and is therefore assessible to targeting by bone seeking pharmaceuticals, providing that affected sites are detectable as "hot spots" on a diagnostic bone-scan. Samarium-153 ethylene diamine tetramethylene phosphonate (Sm-153-EDTMP), in which a radio-isotope emitting beta-radiation (Sm-153) is coupled to a bone-seeking phosphonate (EDTMP), has been employed in this context [92-95]. The standard activity of Sm-153-EDTMP can be increased up to 30-fold with peripheral-blood progenitor cell support, thereby allowing the delivery of substantial radiation doses to osteoblastic lesions.[96-98] Although osteosarcoma is believed to be relatively radioresistant, the total focal dose achieved may delay local progression or, together with external beam radiotherapy, even achieve permanent local tumor control in patients with surgically inaccessible tumors [93].

Unique histological changes in lung metastases of osteosarcoma, namely peripheral fibrosis surrounding the tumor, inflammatory cell infiltration and neovascularization, have been reported for patients who were treated with liposome-encapsulated muramyl tripeptide phosphatidylethanolamine (L-MTP-PE), a biologic response modifier [99]. Animal studies have shown L-MTP-PE to be effective in canine osteosarcoma[100] and patients with relapsed osteosarcoma who received L-MTP-PE therapy had a significant prolongation in time to second relapse in one trial [101]. Interpreting the results of a recent, large POG/CCG-Intergroup evaluating the possible role of L-MTP-PE given in additon to chemotherapy as part of first line treatment against localized extremity osteosarcoma is difficult, as a second randomization – for or against ifosfamide – may have interfered with th eL-MTP-PE question. Nevertheless, the results of the trial have been interpreted as positive by some, as patients randomized to L-MTP-PE did better than others in some analyses [56, 56a].

Interest in the value of interferon-a in osteosarcoma has continued since in vitro effects on osteosarcoma cells were demonstrated more than twenty years ago. Observations since have consistently supported a growth inhibiting effect on osteosarcoma both in cell lines and animal models[102-104]. Two of seventeen patients with advanced bone sarcoma experienced a partial response in a phase II study [105]. Results of a Scandinavian pilot series of adjuvant interferon-a without chemotherapy, where 70 patients were treated with a daily subcutaneous and, with a median follow-up of 12 years, the observed 10-year metastases-free and sarcoma specific survival rates were 39% and 43% [106, 106a] make this agent an attractive target for phase III trials. The European and American Osteosarcoma Study EURAMOS1 is currently evaluating the efficacy of maintenance treatment with pegylated interferon-a following standard chemotherapy in a prospective, randomized trial.

Interleukin 1-a, another cytokine, has been reported to improve the effects observed with etoposide in patients with relapsed osteosarcoma [107]. Some investigators believe that the expression of HER2/erbB-2 may correlate with survival in osteosarcoma [108]. Even though recent evidence suggests that true HER2/neu gene expression may be absent in the disease[109], a phase II trial with the recombinant humanized anti-HER2 antibody trastuzumab (herceptin) is currently under way in the United States.

Combination chemotherapy in resectable disease

Compared to historical controls which relied on surgery only [20], the additional use of polychemotherapy resulted in dramatically improved survival rates in the late 1970s and early 1980s [41-43]. While this was taken as sufficient proof of principle by most investigators, a minority of clinicians still doubted whether chemotherapy was necessary. Based on the results of cohort studies from the Mayo Clinic, they hypothesised that progress might rather have been due to "changing biology" of the

disease, to improvements in surgical techniques or to improved imaging quality [110-113]. This prompted several North American institutions to perform a multiinstitutional study (MIOS) in which an adjuvant polychemotherapy regimen of high-dose methotrexate, doxorubicin, cisplatin, and BCD was compared with a wait and watch strategy. Not surprisingly to most, the results of the MIOS trial demonstrated a convincing superiority of adjuvant chemotherapy over observation. At two and six years, actuarial relapse-free survival was 17% and 11% in the control group and 66% and 61% in the adjuvant chemotherapy group. Even though most patients of the control group went on to receive chemotherapy after relapse, a survival advantage favoring those patients treated with immediate adjuvant chemotherapy was also apparent [18,114]. There has been no further discussion about the necessity or efficacy of chemotherapy for resectable osteosarcoma.

Currently, osteosarcoma is treated with regimens in which a number of cytostatic agents are combined. Most recent protocols have been based upon high-dose methotrexate, doxorubicin, cisplatin, and/or ifosfamide (table 2) [18,41-60]. After more than two decades of experience with these agents and numerous clinical trials, the exact role of each of the drugs and the optimal way in which they are to be combined and delivered are still being debated, as is the potential benefit of additional drugs.

Results of a metaanalysis performed on several polychemotherapy studies from the 1980s suggested that doxorubicin dose intensity is an important determinant of favorable outcome for osteogenic sarcoma.[115] Some investigators believe that a doxorubicin/cisplatin combination may be as efficacious as other, more complex regimens[47,49]. However, many published osteosarcoma trials include patients who have died from anthracycline cardiomyopathy. Recent protocols have therefore include measures aimed towards reducing doxorubicin cardiotoxicity, such as the use of the cardioprotectant dexrazoxane [116] or administering the drug by continuous infusions [117]. While the results of sequential studies by American and European groups suggest that there is no major loss of efficacy, no controlled studies evaluating whether cardiotoxicity can be reduced without influencing efficacy have been reported.

The use of methotrexate against osteosarcoma is still an area of controversy, even though some osteosarcomas definitely show very marked responses to high-dose treatment [30,46,118]. The superiority of high-dose over low-dose methotrexate has been demonstrated in a randomized trial from Italy [45]. Some investigators believe that individual parameters of methotrexate pharmacokinetics correlate with efficacy. It has been suggested that higher peak serum levels [119-121], higher concentrations after 24 and 48 hours [46], or a higher area under the curve [122] may correlate with enhanced methotrexate activity. A comparison of several polychemotherapy trials which included high-dose methotrexate at doses higher than 7.5 g/m^2 concluded that its dose intensity seemed to be a major factor in predicting outcome[123], but this conclusion may have been biased by an inappropriate methodology [124]. In contrast to the proponents of high-dose methotrexate, the European Osteosarcoma Intergroup has claimed that its incorporation into doxorubicin/cisplatin based protocols may not lead to improved results, but may even compromise efficacy [47]. Such a conclusion may have to be questioned, however, as the overall success rate in both arms of the randomized trial EOI 80831 was comparatively low, doxorubicin and cisplatin dose intensities were lower in the methotrexate arm of the trial, and the chosen methotrexate dose of 8 g/m^2 could have been suboptimal. Given the relative lack of myelotoxicity and the resulting ability to schedule methotrexate at times when other, more myelotoxic agents cannot be administered, most groups continue to incorporate high dose methotrexate into their osteosarcoma protocols.

Based on the results of sequential trials, the German-Austrian Swiss COSS-group has reported that adding ifosfamide to doxorubicin, high-dose methotrexate, and cisplatin may be associated with improved response and survival rates[51]. Results from sequential studies performed at the Rizzoli Institute in Bologna also suggested that the response rate might be higher if ifosfamide is added to the other three drugs. However, no correlation between the preoperative use of ifosfamide and overall or

event-free survival could be detected in that series [54]. The only prospective randomized study evaluating the addition of standard dose ifosfamide to a regimen of high-dose methotrexate, doxorubicin, and cisplatin, the Pediatric Oncology Group/Children's Cancer Group intergroup trial INT 0133, could not demonstrate that the addition of ifosfamide improved outcome [56]. The results of that trial, however, need to be interpreted with caution: A second, parallel randomization for or against the addition of liposomal muramyl tripeptide (L-MTP-PE) may have interfered with the ifosfamide question. Also, cisplatin was only included postoperatively in the ifosfamide arm.

Following reports of efficacy in chemotherapy naive as well as pretreated osteosarcoma [125], a combination of bleomycin, cyclophosphamide, and actinomycin D (BCD) enjoyed rather widespread use in the early days of chemotherapy[41,43]. A randomized trial comparing cisplatin with BCD found no benefit for one or the other [43]. BCD was later largely abandoned when a subsequent phase II trial in found no activity [126]

As mentioned above, metaanalyses have suggested that the dose-intensity of treatment, particularly doxorubicin [115] or high-dose methotrexate [123], might correlate with outcomes. The results presented in several recent reports argue against an effect of increasing dose intensity above that normally encountered in modern protocols. A review of 917 consecutive patients from COSS trials could not detect a correlation of either higher overall treatment intensity or of the dose intensity of individual agents with either overall or event-free survival [126a]. Similarly, while an increase of dose intensity could be reached by adding G-CSF to a doxorubicin/cisplatin regimen, the event free survival rates were not altered by the addition of G-CSF in a recent, randomized trial of the European Osteosarcoma Intergroup [58, 58a].

Scheduling chemotherapy: The role of preoperative induction chemotherapy

Parallel to the development of efficacious chemotherapy protocols, surgeons were starting to replace amputation of the tumor-bearing extremity by limb-salvage surgery.Today, the defects left by limb-salvage procedures can be reconstructed by modular prostheses which are assembled to fit in the operating theater. In the early days of limb-salvage surgery, however, only custom-fitted implants were available, and these took several weeks to build. Surgery therefore had to be delayed considerably, if the limb was to be salvaged. In an attempt to bridge the resulting time period, preoperative chemotherapy was introduced by Rosen and coworkers from New York to shrink the primary tumor and to allow complete surgical removal without amputating the involved limb [127]. It was suggested that preoperative chemotherapy might offer additional advantages, including the early use of systemic therapy to eradicate distant microfoci of disease, evaluation of the effect of chemotherapy on the primary tumor, and providing time for the surgeon to plan resection surgery [128].

Historically, the introduction of preoperative chemotherapy and the dramatic improvements of outcome of osteosarcoma patients occurred at the same time, approximately 30 years ago. Among the proponents of the approach, this was taken as a strong argument for their theory that an immediate start of chemotherapy could lead to superior results compared to one that was delayed by surgery and postoperative wound healing. On the other hand, there was concern about local disease progression and, even more so, continued dissemination of new metastases over the weeks and months in which the tumor was left in place, at least in cases where the osteosarcoma was chemoresistant. In addition, it was felt that delayed wound healing in individuals already immune compromised by chemotherapy might compromise the dose intensity of postoperative chemotherapy, thereby reducing a patient's chances for cure.

As most modern osteosarcoma-studies include preoperative chemotherapy while those in which only postoperative therapy was used were the earliest trials, comparing their results would mean having to introduce a serious bias. Only a single, relatively small prospective randomized trial has focussed on the question whether pre-plus postoperative chemotherapy leads to superior cure rates compared to primary surgery followed by the same chemotherapy regimen postoperatively. In this Pediatric Oncology Group trial, treatment results did not differ between the

two arms.[59] Similarly, neither the Cooperative Osteosarcoma Study Group, in a large retrospective comparison of 157 patients with primary surgery and 1451 with preoperative chemotherapy[21], nor Memorial Sloan Kettering Cancer Center, analyzing 279 patients treated either way[48], were able to detect a survival difference between both approaches.

Preoperative chemotherapy offers the unique opportunity to evaluate the efficacy of the chosen drug combination against a malignancy on an index lesion. Other than most tumors, osteosarcoma do not reliably shrink even if chemotherapy is highly effective. This is due to the presence of an osteoid matrix, which remains in place long after the tumor cells have been destroyed. The evaluation of response to preoperative chemotherapy must therefore be performed histologically on the resected tumor specimen. An experienced pathologist must assess which percentage of the tumor still contains viable malignant cells. Various grading systems are used, with those developed by Huvos et al [1] and Salzer-Kuntschik et al [129] featuring most prominently. Most investigators would define a good response to preoperative chemotherapy as less than 10 percent viable tumor.

Soon after the custom of delaying surgery by preoperative chemotherapy had been introduced, it became obvious that the degree of histological response of the primary tumor was closely related to the risk of systemic, metastatic recurrence[41,43]. Ever since then, a pivotal prognostic importance of tumor response has been detected in a wide variety of prospective studies employing varying chemotherapy regimens[44,45,47-51]. Response to preoperative chemotherapy was confirmed as the most important prognostic factor in respectable osteosarcoma in a metaanalysis of prospective trials [130] and in a review of 1.702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols [21]. Increasing the intensity of preoperative chemotherapy may increase the proportion of patients showing a good histological response, but may not result in improved overall survival [54,131]. While osteosarcomas are usually characterized as responding either well or poorly, response must not be misinterpreted as an all-or-nothing phenomenon, but should be understood as a gradual effect. Even patients with more than 50% viable tumor cells remaining still seem to have a better prognosis than patients without any response at all [21] or those not receiving chemotherapy [18,20].

Immediately after it became clear that a poor response to a particular preoperative regimen implied a poor prognosis, it was tried to improve outcomes by modifying postoperative chemotherapy for poor responders. The first report that such a "salvage" approach was successful again came from Rosen et al from Memorial Sloan-Kettering Cancer Center, who claimed that poor responders reached the same prognosis as good responders if postoperative chemotherapy was modified to include new agents [42]. This message was taken up enthusiastically by the osteosarcoma community, and a multitude of uncontrolled trials employing salvage chemotherapy for poor responders followed. To everyone's disappointment, it soon became apparent that salvage chemotherapy, at least of the type used in the reported trials, did little to nothing to improve the poor prognosis of poor responders **(Table 2)**. The failure of salvage chemotherapy was especially apparent in a trial from the Cooperative Osteosarcoma Study Group, where the effective but toxic agents doxorubicin and cisplatin were deliberately omitted from preoperative chemotherapy and only used postoperatively and only in poor responders[44]. Finally, a reanalysis of the Memorial cohort for whom successful salvage had initially been claimed [42] failed to demonstrate any positive effect of altering chemotherapy postoperatively [48]. Therefore, as of now, there is no proof that poor responders will benefit from postoperative treatment modifications. One questionable exception to the failure of salvage therapy is high-dose ifosfamide, for which some efficacy was claimed based on results from an uncontrolled Italian study [53]. The European and American Osteosarcoma Study EURAMOS1 is currently assessing salvage chemotherapy with high-dose ifosfamide in a prospective, randomized manner [53a].

Surgery is still the treatment of choice for local tumor control in osteosarcoma. It is strongly recommended to refer patients to specialized

surgeons, as surgical errors can irrevocably compromise a patient's chances for limb salvage or even survival.

Over the past two decades, there has been a major shift away from amputations towards limb-salvage surgery, and surgeons have stressed that pretreated tumors are better demarcated against the surrounding tissues and easier to operate[24]. Amputation rates of less than 20% have been reported for patients treated in recent years [21,131]. Chemotherapy alone does not even come close to controlling the primary tumor[28], but it may contribute to local control in operated patients[23,27]. Altogether, in extremity osteosarcoma, local failure rates have been as low as 5 to 8 percent in several large series [23,27,132]. Inadequate surgical margins are the most important risk factor for local failure[23]. A good tumor response will contribute to the safety of surgery. Several groups have found local failure rates which were approximately three times lower for good as opposed to poor responders to preoperative chemotherapy [23,27]. Response and margins interact, and the local failure rate becomes excessive if inadequate margins and poor response come together.[23] Limb-salvage surgery may be associated with increased local failure rates in poor responders[27], implying that margins are sometimes not as wide as assumed during surgery. Surgeons should therefore try to be aware of the viability of the tumor they are going to encounter. Histological response to neoadjuvant chemotherapy can quite reliably be predicted by a variety of techniques, such as dynamic MRI [133], quantitative bone scans [134], or positron emission tomography [135,136].

Conclusions

Osteosarcoma is a curable disease. Surgery and chemotherapy are the mainstays of treatment. Effective chemotherapy regimens have been based on combinations including several of the drugs doxorubicin, cisplatin, high-dose methotrexate, and ifosfamide. Delaying surgery with preoperative chemotherapy neither improves nor reduces a patients survival expectancy, but allows to prepare for safe limb-salvage surgery. In addition, it offers the unique opportunity to assess the most important prognostic factor in resectable osteosarcoma, histological tumor response. The successful treatment of patients with osteosarcoma requires close cooperation within an experienced interdisciplinary team including oncologists, surgeons, pathologists, and radiologists. Therapy should be performed in specialized centers able to provide the full spectrum of care. As in other rare malignancies, treatment should be administered within the scope of prospective multicenter trials. Therapy must include complete surgical removal of all detectable tumor foci as well as multiagent chemotherapy. The chemotherapy regimen should include several of the following four drugs: doxorubicin, high-dose methotrexate, cisplatin, and ifosfamide. Preoperative plus postoperative as opposed to purely postoperative chemotherapy may not be associated with survival benefits, but should be preferred because it allows to prepare for safe surgery. The choice of the definitive surgical procedure should be influenced by the anatomical site of the primary tumor, by its relationship to neighboring structures such as vessels and nerves, by the age of the patient and his growth potential, and by the anticipated response of the tumor to preoperative chemotherapy. The risk of both local and metastatic recurrence is strongly dependent on the response of the tumor to preoperative chemotherapy. At present, there is no compelling evidence that alterations of postoperative treatment can improve the prognosis for patients with a poor response to preoperative induction chemotherapy. Centers in participating European countries and North America are strongly encouraged to participate in the EURAMOS trial, which addresses the question of salvage chemotherapy for poor responders in a randomized, controlled fashion. A major problem yet waiting to be solved is the dismal outlook for patients with unresectable or relapsed osteosarcoma. Novel approaches are needed to improve their prognosis.

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Preventing Invasive Bacterial Infection in Neutropenic Patients with Cancer

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Introduction

Children receiving myelosuppressive chemotherapy are at risk for febrile neutropenia, invasive infections and infection-related mortality. Risks for these different outcomes vary according to several factors including the depth and duration of neutropenia[1], presence of co-morbidities[2], and other clinical and laboratory markers.[3-6] Children at high risk of infectious morbidity include those with acute myeloid leukemia (AML), relapsed acute lymphoblastic leukemia (ALL) and those receiving myeloablative hematopoietic stem cell transplantation (SCT). For example, pediatric AML treatment-related mortality ranges from 4 to 11%[7], with most of this mortality attributed to infection. An infection-related mortality of 25/341 (7.3%) was reported for children treated according to MRC-10.[8] The most recent Children's Cancer Group (CCG) AML study, CCG 2961 reported 1,007 blood borne infection among 553 children receiving 1,243 courses of chemotherapy.[9]

Infectious outcomes including febrile neutropenia and invasive infections are clinically important outcomes that impact on costs, quality of life and mortality. Thus, interventions to prevent infectious outcomes have been tested in hundreds of randomized controlled trials (RCTs). This review will focus on two specific interventions to prevent invasive bacterial infections, namely prophylactic hematopoietic colony-stimulating factor (CSF) and prophylactic antimicrobial administration.

Colony stimulating factors

Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are hematopoietic CSFs that decrease the duration and severity of neutropenia in adults and children who receive chemotherapy for cancer.[10,11] However, elucidation of a target population who might particularly benefit from CSFs in terms of clinically important infectious outcomes continues to be refined. Initially, three large RCTs of adults receiving chemotherapy for cancer were particularly influential in early guideline development and recommendations for CSF utilization. These trials demonstrated reductions in the risk of febrile neutropenia from 77% to 40%[12], 53% to 26%[13] and 44% to 23%[14] with CSF administration. In other words, these trials demonstrated that in adults receiving moderately intensive chemotherapy that resulted in a frequency of febrile neutropenia of 44% to 77% in the control arms, prophylactic G-CSF was associated with an approximately 50% reduction in febrile neutropenia.[12-14] On the basis of these studies, early guidelines published by the American Society of Clinical Oncology (ASCO) suggested that CSFs be used as primary prophylaxis (before onset of neutropenia or febrile neutropenia) when the expected incidence of febrile neutropenia is 40% or more.[11,15,16]

Subsequently, several systematic reviews have examined the question of whether CSFs are useful for cancer patients in the prophylactic setting. Lyman et al. conducted a meta-analysis of trials of prophylactic G-CSF in patients receiving dose-intensive chemotherapy for either solid tumors or malignant lymphomas.[17] Eight randomized trials were identified. The use of G-CSF was associated with a reduction in febrile neutropenia, with an odds ratio (OR) of 0.38 (95% confidence interval [CI] 0.29 to 0.49; P=.001). G-CSF also was associated with a reduction in documented infections, with an OR of 0.51 (95% CI 0.36 to 0.73; P=.001). However, there was no reduction in infection-related mortality with an OR of 0.60 (95% CI 0.30 to 1.22; P=.2).[17]

In addition to the systematic review conducted by Lyman, there also have been meta-analyses

performed within specific diagnostic sub-groups. For example, Bohlius et al. performed a meta-analysis of prophylactic G-CSF and GM-CSF in patients with malignant lymphoma.[18] Eleven randomized trials were included. In this review, CSFs reduced the risk of neutropenia, with a relative risk (RR) of 0.64 (95% CI 0.55 to 0.75). In addition, CSFs reduced the risk of febrile neutropenia (RR 0.74, 95% CI 0.62 to 0.89) and microbiologically documented infection (RR 0.74, 95% CI 0.64 to 0.85). However, CSFs did not affect either overall mortality during chemotherapy (RR 1.21, 95% CI 0.70 to 2.10) or infection-related mortality (RR 2.07, 95% CI 0.81 to 5.34). Furthermore, CSFs were not associated with a benefit in terms of overall survival at an average observation time of four years (hazard ratio [HR] 0.97, 95% CI 0.81 to 1.17).[18] These authors later updated their meta-analysis to include 12 randomized trials; the results of this meta-analysis were qualitatively similar to those of their previous publication.[19]

In another population, Berghmans and colleagues examined 12 RCTs of prophylactic CSFs in small cell lung cancer.[20] CSFs were not associated with improved survival.[20] Adams et al. reviewed cost-effectiveness models of prophylactic G-CSF use in this population. The frequency of febrile neutropenia in the control arm required to result in cost saving associated with G-CSF use ranged from 35 to 70%.[21]

In another systematic review, the results of 16 RCTs of prophylactic CSFs in pediatric cancer patients were synthesized.[22] The mean rate of febrile neutropenia in the control arms was 57% (range 39 to 100%). Using a random effects model, CSFs were associated with a 20% reduction in febrile neutropenia, with a rate ratio of 0.80 (95% CI 0.67 to 0.95; P = .01), and a decrease in hospitalization length, with a weighted mean difference (WMD) of –1.9 (95% CI –2.7 to -1.1 days; P < 0.00001). CSF use also was associated with reduction in documented infections (rate ratio 0.78, 95% CI 0.62 to 0.97; P = .02) and reduction in amphotericin B use (rate ratio 0.50, 95% CI 0.28 to 0.87; P = .02). There was no difference in duration of parenteral antibiotic therapy (WMD –4.29, 95% CI -10.60 to 2.02 days; P = 0.2) or infection-related mortality (rate ratio 1.02, 95% CI 0.34 to 3.06; P = 0.97).[22] Using a stratified analysis, this systematic review suggested that the effects of G-CSF and GM-CSF were similar. However, a secondary analysis using the same data found that G-CSFs may be associated with more clinical benefits. Specifically, this second meta-analysis found that there was a 90% probability that G-CSF was more effective than GM-CSF in reducing the rate of febrile neutropenia. G-CSF also was associated with a 4.8 day greater decrease in duration of parenteral antibiotic therapy compared to GM-CSF and there was a 98% probability that G-CSF was better than GM-CSF with respect to this outcome.[22,23]

A second pediatric meta-analysis of prophylactic CSFs was conducted in children with ALL.[24] Six studies of 332 children were included. The use of CSFs reduced the risk of febrile neutropenia (rate ratio 0.63, 95% CI 0.46 to 0.85; P=.003), duration of hospitalization (WMD -1.58, 95% CI -3.00 to -0.15; P=.03) and number of infections (rate ratio 0.44, 95% CI 0.24 to 0.80; P=.002).[24]

Up until this point, given that clinically important benefits with the prophylactic administration of CSFs had been demonstrated only following administration of more intensive chemotherapy, and since recipients of SCT almost universally experience febrile neutropenia, we postulated that this group of patients might particularly benefit from prophylactic CSF administration. Thus, we conducted a meta-analysis of prophylactic CSF administration in the SCT setting. There were 34 included studies based on pre-defined inclusion criteria. CSFs reduced the risk of documented infections (RR 0.87, 95% CI 0.76 to 1.00; P=.05) and duration of parenteral antibiotics (WMD -1.39 days, 95% CI -2.56 to -0.22; P=.02) but did not reduce infection-related mortality (RR 0.76, 95% CI 0.41 to 1.44; P=.4).

In summary, the results of many RCTs and meta-analyses have found a consistent benefit of prophylactic CSF administration in preventing febrile neutropenia and invasive bacterial infections. So far, a reduction in infection-related mortality has not been demonstrated. The target population who would benefit most from prophylactic CSFs remains to be defined. Although intuitively recipients of SCT may have been expected to benefit most from CSF administration, the data have not supported this hypothesis. It is possible that those with extreme

degrees of myelosuppression benefit less than those with more intermediate risks of febrile neutropenia (i.e. between 40% and 80%). However, such a hypothesis remains to be confirmed.

Antibiotic prophylaxis

A second intervention of interest has been prophylactic administration of antibiotics to prevent invasive bacterial infections. Current guidelines do not recommend routine antibiotic prophylaxis in cancer patients.[25] However, a survey conducted by the Japan Adult Leukemia Study Group in 2001 that included 196 hospitals found that 38% of physicians used an oral quinolone for antibacterial prophylaxis in patients with leukemia.[26] While the bulk of RCTs examining this question extend back over the last three decades, recent RCTs and meta-analyses have continued to add to the controversy.

Most interest has been directed at fluoroquinolones because of their broad antimicrobial spectrum, preservation of the anaerobic flora of the alimentary tract, high concentration in the feces, systemic bactericidal activity, good tolerability and lack of myelosuppression.

The first two meta-analyses to address this topic both demonstrated a reduction in Gram negative bacteremia but no affect on infection-related mortality.[27,28] More specifically, Engels and colleagues included 18 studies of quinolone prophylaxis and showed a reduction in Gram negative infections (RR 0.21, 95% CI 0.12 to 0.37), microbiologically documented infections (RR 0.65, 95% CI 0.50 to 0.85), total infections (RR 0.54, 95% CI 0.31 to 0.95) and fevers (RR 0.85, 95% CI 0.73 to 0.99).[27] Cruciani et al. included 19 studies and similarly showed fluoroquinolones prevented Gram negative bacteremia (OR 0.09, 95% CI 0.05 to 0.16). Neither study demonstrated a reduction in infection-related mortality

Two subsequent meta-analyses of prophylactic antibiotic administration did show reductions in infection-related mortality. First, van de Wetering et al. examined 21 trials of oral prophylaxis antibiotics in neutropenic afebrile oncology patients.[29] Prophylaxis reduced bacteremia (OR 0.48, 95% CI 0.34 to 0.66) and Gram negative bacteremia (OR 0.39, 95% CI 0.24 to 0.62). Most importantly, this analysis demonstrated a reduction in infection-related mortality, with an OR of 0.56 (95% CI 0.34 to 0.96). The most recent trial included in this systematic review was published in 2002.

Finally, Gafter-Gvili et al. conducted the largest meta-analysis of prophylactic antimicrobial administration in cancer patients and specifically examined the efficacy of administration on survival.[30] They included randomized trials comparing antibiotic therapy with placebo, no intervention or another antibiotic for prophylaxis of bacterial infections in neutropenic patients. The primary outcome was all-cause mortality by the end of follow-up defined in each study. Secondary outcomes included infection-related death, febrile episodes, clinically documented infection, microbiologically documented infection, bacteremia, adverse effects and emergence of resistant bacteria. In total, 95 studies performed between 1973 and 2004 were included and 9,283 patients were randomized. Fifty trials compared a prophylactic antibiotic with placebo or not intervention, of which 17 trials evaluated fluoroquinolones. Prophylaxis was initiated either when the patient became neutropenic (17 trials) or with the initiation of chemotherapy (78 trials). In all but two trials, prophylaxis was continued until resolution of neutropenia, fever or remission developed, or the patient received a maximum of 6 weeks of treatment Overall, antibiotic prophylaxis decreased the risk of death with a RR of 0.67 (95% CI 0.55 to 0.81). In addition, prophylaxis was associated with reductions in infection-related death (RR 0.58, 95% CI .55 to .81), fever (RR 0.79, 95% CI 0.75 to 0.82), clinically documented infections (RR 0.64, 95% CI 0.60 to 0.71), microbiologically documented infections (RR 0.54, 95% CI 0.49 to 0.60), Gram negative infections (RR 0.39, 95% CI 0.32 to 0.46), Gram positive infections (RR 0.42, 95% CI 0.35 to 0.50), and bacteremia (RR 0.052, 95% CI 0.46 to 0.59). Prophylaxis was not associated with increased fungal infection (RR 1.07, 95% CI 0.83 to 1.37). However, prophylaxis was associated with more adverse events (RR 1.57, 95% CI 1.33 to 1.86). The most recent included trial was published in 2003.

An important question relating to these meta-analyses is whether the results are applicable today given that most of the included studies were conducted over a decade ago. Over the last three decades, initially Gram negative pathogens predominated as a cause of morbidity and mortality in febrile neutropenic patients. Over time, the incidence of Gram positive infections has increased along with a world-wide increase in the incidence of viridans group streptococcal infection.[31-33] Two recently conducted RCTs have tried to address this issue. In both studies, levofloxacin was the prophylactic agent studied.

Bucaneve and colleagues examined a high risk population who were expected to be neutropenic for greater than 7 days.[34] They assigned 760 consecutive adult patients with cancer to receive oral levofloxacin or placebo from start of chemotherapy until resolution of neutropenia. Antibiotic prophylaxis was associated with a reduction in fever from 85% with placebo to 65% with levofloxacin (RR 0.76, P=.001). In addition, lower rates of microbiologically documented infection, bacteremia and single-agent Gram negative bacteremia were demonstrated. However, mortality was similar, occurring in 10/373 (3%) with levofloxacin versus 18/363 (5%) with placebo (P=.15). Infection-related mortality also was similar, occurring in 9/373 (2%) with levofloxacin versus 14/363 (4%) with placebo.

In contrast, the second recent RCT by Cullen and colleagues examined a low risk population and included those receiving chemotherapy for solid tumors and lymphomas.[35] This study allocated 1,565 subjects to levofloxacin or placebo for 7 days during expected neutropenia. They demonstrated a 4.4% decrease in the prevalence of febrile episodes attributable to infection in the first cycle and a 4.4% decrease in the cumulative incidence of any febrile episode. However, the baseline risk of clinically documented febrile episodes attributable to infection in the first cycle was only 7.9%. Four patients died in each group.

Given these results, it is likely that there is a patient population who is expected to derive meaningful benefit from prophylactic antibiotic administration. However, any potential benefit must be weighed against adverse events, particularly in terms of increasing antimicrobial resistance. Since infections with resistant micro-organisms tend to be associated with worse outcomes, non-discriminate use of antibiotic prophylaxis may cause worse infectious outcomes for later cohorts of patients. Thus, future work will likely focus on identification of a patient population who is most likely to benefit from antibiotic prophylaxis. Furthermore, the optimal period for prophylaxis has yet to be defined. For example, it is likely that prophylaxis could be discontinued upon evidence of bone marrow recovery which would be associated with less antimicrobial administration compared to waiting until resolution of neutropenia.

Summary

The last three decades has seen a plethora of RCTs with the primary aim of reducing infections in neutropenic cancer patients. While CSFs can reduce invasive infections, prophylaxis has not been demonstrated to improve survival. In contrast, prophylactic antibiotics can reduce mortality. However, concerns about whether the results are generalizable to the current microbiological milieu and the potential for antimicrobial resistance have limited its widespread adoption. Future research will likely focus on identifying patient populations more likely to benefit from a particular intervention while balancing costs and issues of drug resistance.

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Radiological staging of primary malignant liver tumors in children

Derek J Roebuck

Staging of primary malignant liver tumors

Several staging systems are potentially applicable to children with primary liver tumors. This paper will concentrate on the 2005 revision of the PRETEXT system, which is almost completely based on imaging findings.

Segmental anatomy

The system of segmental anatomy promoted by Couinaud [1] is widely used throughout the world, with minor modifications. For imaging purposes, the liver is divided into segments by planes defined by the courses of the hepatic veins and the branches of the portal vein. The plane of the right hepatic vein separates the right posterior section (segments 6 and 7) from the right anterior section (segments 5 and 8). The plane of the middle hepatic vein separates the right anterior section from the left medial section (segment 4), and therefore forms the boundary between the right and left lobes of the liver. A plane that runs close to the fissure of the ligamentum teres (essentially the plane of the umbilical segment of the left portal vein) separates the left medial and lateral (segments 2 and 3) sections.

Pretext number

In the 2005 revision of the PRETEXT system, the PRETEXT number is derived by subtracting the highest number of contiguous liver sections that are not involved by tumor from four [2]. For these purposes, pedunculated or other exophytic tumors are considered to involve only the section(s) from which they originate. The only exception to this rule involves tumors that arise from or extend into the caudate lobe (segment 1), which are classified as a minimum of PRETEXT II.

Additional criteria in the PRETEXT system (Table 1)

Additional criteria assess various potential markers of poor prognosis in children with malignant primary liver tumors. The original criteria were involvement of the inferior vena cava (IVC) or hepatic veins (designated **V**), involvement of the portal veins (**P**), extrahepatic abdominal disease (**E**) and distant metastases (**M**). The 2005 revision redefined and extended these criteria, adding involvement of segment 1 (**C**), multifocal liver tumor (**F**), tumor rupture at diagnosis (**H**) and lymph node metastasis (**N**). The intention of these changes was to identify prognostic imaging findings, and in the long term to refine risk stratification.

Imaging techniques

The standard imaging investigations in children with a primary malignant liver tumor are ultrasound (US) and either or both of contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MR) of the abdomen, as well as CT of the thorax. Baseline orthogonal measurements of the largest liver lesion, as well as measurements of the maximum diameter of all target lesions are made, according to the requirements of the RECIST system [3]. Interpretation of imaging studies in these children is often very difficult. In the SIOPEL studies, centralized radiological review is recommended for all patients (www.siopel.org).

Ultrasound (US)

US is the best first imaging test in a child with a suspected abdominal mass, and will therefore be performed in almost all children with liver tumors. It is particularly good for the evaluation of vascular anatomy [4]. This is important in the PRETEXT system because it is the basis of assessment of both segmental involvement (and therefore the PRETEXT number) and involvement of the IVC and hepatic veins (**V**) and the portal veins (**P**). US may be repeated after CT or MR to resolve any difficulties with evaluation of the hepatic and portal veins. It may be helpful if the liver surgeon is present. A sector

Table 1 : Additional criteria in the 2005 revision of the PRETEXT staging system.

Caudate lobe involvement

C1 Tumor arising from or extending into the caudate lobe

C0 All other patients

All **C1** *patients are at least PRETEXT II*

Extrahepatic abdominal disease

E0 No evidence of tumor spread in the abdomen (except **M** or **N**)

E1 Direct extension of tumor into adjacent organs or diaphragm

E2 Peritoneal nodules

Add suffix "a" if ascites is present, e.g. **E0a**

Tumor focality

F0 Patient with solitary tumor

F1 Patient with two or more discrete tumors

Tumor rupture or intraperitoneal hemorrhage at diagnosis

H1 Imaging and clinical findings of intraperitoneal hemorrhage

H0 All other patients

Distant metastases

M0 No metastases

M1 Any metastasis (except **E** and **N**)

Lymph node metastases (must be proved by biopsy in hepatoblastoma)

N0 No nodal metastases

N1 Abdominal lymph node metastases only

N2 Extra-abdominal lymph node metastases (with or without **N1**)

Portal vein involvement

P0 No involvement of the portal vein or its left or right branches

P1 Involvement of either the left or the right branch of the portal vein

P2 Involvement of the main portal vein

Add suffix "a" if intravascular tumor is present, e.g. **P1a**

Involvement of the IVC and/or hepatic veins

V0 No involvement of the hepatic veins or inferior vena cava (IVC)

V1 Involvement of one hepatic vein but not the IVC

V2 Involvement of two hepatic veins but not the IVC

V3 Involvement of all three hepatic veins and/or the IVC

Add suffix "a" if intravascular tumor is present, e.g. **V3a**

or curved linear array transducer is used for the first part of the examination [4]. This allows confirmation of hepatic origin of the tumor and is useful for measuring the size of large tumors. High-frequency linear array transducers are often used to assess the portal and hepatic venous systems, and for accurate measurement of small lesions. Color Doppler imaging and pulsed wave Doppler examination may also be helpful to identify intravascular tumor growth [4-6].

Abdominal CT

Where possible, MR is now preferred to CT [4], but in many centers CT is still more easily available. Oral contrast is optional [4]. If images are obtained before intravenous contrast they should be limited to the liver [4]. Following administration of intravenous contrast (using a pump injector), images of the abdomen and pelvis are obtained in the portal venous phase. Newer (multi-detector) CT scanners minimize the need for sedation and allow multiplanar images to be reconstructed.

Chest CT

This is mandatory to look for pulmonary metastases, which are common in both hepatoblastoma and hepatocellular carcinoma. Unfortunately there are no standard diagnostic criteria for lung metastases on CT, and central radiological review (see above) may be helpful. Basal atelectasis is very common in sedated or anesthetized children, and this may mimic or obscure metastases [4]. Careful anaesthetic technique may overcome this problem to some extent [4;7].

Magnetic resonance imaging (MR)

There have been and will continue to be rapid advances in MR technology and imaging sequences. This makes it difficult to formulate precise recommendations for imaging protocols. Selection of an appropriate coil and the use of respiratory triggering are important to optimize image quality [4]. Inversion recovery images are useful because of their very high signal-to-noise ratio. Spin echo and inversion recovery sequences are commonly obtained [4]. Volume interpolated spoiled gradient echo sequences give excellent images following administration conventional gadolinium-based contrast agents [4].

Table 2 : Risk stratification in hepatoblastoma for current SIOPEL studies.

High risk
A patient with any of the following:
serum alpha-fetoprotein <100 μg/L
PRETEXT IV
E1, E1a, E2, E2a
H1
M1
N1, N2
P2, P2a
V3, V3a
Standard risk
All other patients

Other imaging techniques

Angiography is now obsolete in the evaluation of primary malignant liver tumors. Bone scintigraphy is recommended in hepatocellular carcinoma, because skeletal metastases are common, but not in hepatoblastoma. In fact, bone scintigraphy in children with hepatoblastoma may produce false-positive studies because of paraneoplastic osteopenia related to abnormal bone metabolism, which is present in many children [4;8].

Current SIOPEL trials

SIOPEL currently stratifies children with hepatoblastoma into standard and high risk categories **(Table 2)**. At the time of writing, standard risk patients are eligible for SIOPEL 3, but the new SIOPEL 6 trial is expected to open in late 2006. This will be a randomized phase II trial of the efficacy of sodium thiosulfate in reducing ototoxicity associated with cisplatin monotherapy. Children with high risk hepatoblastoma are eligible for SIOPEL 4, a non-randomized phase II trial of intensified preoperative chemotherapy. SIOPEL 5, a non-randomized study which adds antiangiogenic therapy and post-operative metronomic chemotherapy to the standard PLADO chemotherapy, is now open for children and young adults with non-cirrhotic hepatocellular carcinoma and fibrolamellar carcinoma. Further information on SIOPEL trials is available at www.siopel.org.

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Treatment of hepatoblastoma

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For the International Childhood Liver Tumour Strategy Group of the International Society of Paediatric Oncology – SIOPEL Group

The treatment of hepatoblastoma (HB) is based on systemic (CT) and surgery (S). The goal of the therapeutic strategy is to remove completely the tumour; in fact only those children who achieved the complete resection may hope in a definitive cure. After surgery alone the vast majority of children are expected to suffer of tumour recurrence.

In the early '80s the 5-overall-survival (OS) of children affected by HB was estimated to be on the 30% range. From the late '80s with the introduction of Cisplatin (CDDP) based CT regimen a dramatic change in the prognosis of these young patients was started to be documented. The first reports on the effect of this drug on HB regarded relapsing or resistant HB to the conventional CT of those years, represented by the combination of cyclophosphamide, vincristine (VCR) and 5-fluouracile (5-FU). Those first experiences reported significantly tumour volume reduction in response to CDDP exposure. Counting on the fat that approximately only 30% of the HB at diagnosis is respectable and based on those preliminary data, clinical investigators started to move CDDP-based treatment, up-front, meaning soon after diagnosis. The effect of CDDP in "making the tumour smaller" and resectable was further confirmed. It was also a common surgeons' feeling that the tumour after CDDP exposure was more compact, less bloody and more demarcated from the adjacent healthy liver parenchyma.

In Europe in the early nineties, the concept of deferring definitive surgery for childhood solid tumours after an initial course of CT (in order to make subsequent definitive surgery more likely radical and safer), was already entered into the standard clinical practice by the trials on Wilms tumour of International Society of Paediatric Oncology (SIOP). Based on the above mentioned data and on the Wilms tumour experience, the International Childhood Liver Tumour Strategy Group of SIOP – SIOPEL group - decided to design it first cooperative large multi-institutional trials on a treatment strategy based on pre-operative CT followed by definitive surgery. Since then, this strategy has always marked all the subsequent clinical trials run by this group. Differently, in North America, the clinical investigators of the Children's' Cancer Study Group (CCSG), of the Paediatric Oncology Group (POG) and more recently of the Children's' Oncology Group (COG) maintained the treatment concept of initiating treatment for childhood HB always attempting definitive surgery (and thus of using CT afterwards). In the American trials only those children bearing a clearly unresectable tumour at diagnosis started treatment with systemic CT. In brief since now two treatment philosophies for the treatment of HB exist: the one used by the SIOPEL group based on primary CT followed by definitive surgery (and also by a short course of post-surgical CT) and the other used by the North American Group based on primary surgery followed by CT.

Despite these different approaches, both those two major study groups project 5-year OS well above 70%. The combinations CDDP/ Doxorubicin and CDDP/VCR/5-FU represent the referring CT for the SIOPEL and the North American groups respectively.

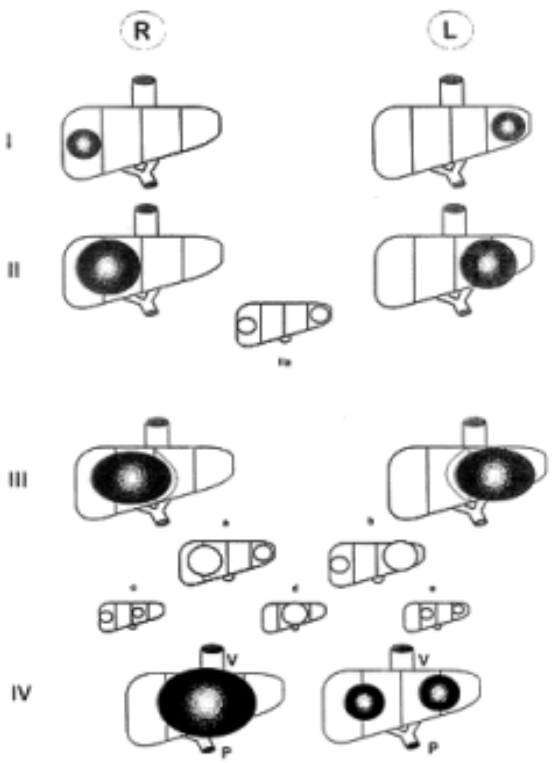
The present clinical research strategy aims to refine therapy according to well established prognostic factors. According to the North American experience, the presence of distant metastases and of macroscopical residual

disease after surgery represent the most important negative prognostic factors. Furthermore, in their trials, children with a pure fetal HB microscopically completely removed, had such an excellent prognosis that for this small subset of patients (around 5% of all HB) no post-operative CT is presently recommended. The other most important prognostic factors outlined by the American investigators are represented by the magnitude of the alpha-fetoprotein (AFP) decrease in response to therapy. Those patients in fact who experience a significant decreased of the AFP during the initial phase of CT seems to have an excellent prognosis.

In the SIOPEL group study the used of a therapeutic strategy based on pre-operative CT allowed to assess the prognostic impact of some clinical and histological characteristics before any therapeutic act. Just for the purpose of describing tumour extension before any therapeutic intervention, the SIOPEL group elaborated an *ad hoc* tumour extension evaluation system called PRETEXT - pre treatment extent of disease evaluation system – **(Fig 1)**. In the PRETEXT system, the liver is divided into four sectors - an anterior and a posterior sector on the right and a medial and a lateral sector on the left. In this way, four PRETEXT categories are identified. Tumors are classified as PRETEXT I when three adjoining sectors are free and only one is involved by the tumor; as PRETEXT II when two adjoining sectors are free and two involved; as PRETEXT III when just one sector is free and three involved (or two non-adjoining sectors are free) and PRETEXT IV when there are no tumor-free sectors. Intra-abdominal extension of the tumor beyond the liver is indicated by: "V" in the case of extension into the vena cava and/or all three hepatic veins; "P" in the case of extension into the main and/or both left and right branches of the portal vein; "E" in the case of extra-hepatic extension other than "P" and "V" ; and "M" when distant metastases are detected.

In the first trial run by the SIOPEL group – SIOPEL I - the extent of pre-treatment intra-hepatic disease, as defined by the PRETEXT system and the presence of lung metastases were identified as prognostic factors for 5-years event-free survival (EFS); but in multivariate analysis the PRETEXT category was the only statistically significant prognostic factor for 5-year OS. Based on these findings, the SIOPEL group decided for his subsequent trial – SIOPEL 2 - to stratify treatment according to the intra-hepatic extension of the disease (as expressed by the PRETEXT system) and the presence of lung metastases. More precisely it was decided to investigate if some therapy intensification would ameliorate the prognosis of those children affected by an HB extending to all 4 hepatic sectors (PRETETX IV) and/or with metastases and if it would be possible to reduce therapy without jeopardizing the overall outcome, to those patients affected by a tumour completely confined to the liver and involving at the most three hepatic sectors. For the purpose of the trial the first group of HBs were called "high-risk" (HR)-HB and the second "standard risk" (SR)–

Fig.1: PRETEXT grouping system



Legend: *V= extension into the vena cava and/or all three hepatic veins; P =extension into the main portal vein and/or both left and right portal branches; E, extrahepatic excluding extrahepatic V or P (rare); M, distant metastases;*

HB. Within the HR group also patients with intra-abdominal extension of the tumour beyond the liver were included despite definite evidences that these factors aggravated the prognosis were missing. Thanks to the contribution of the German Study Group on HB in the early nineties also the AFP value at diagnosis emerged as a significant prognostic factors. More precisely those children presenting with an AFP of less than 100 hgr/L seemed to have a very unfavourable outcome. Thus, in the SIOPEL2 trial also those latter tumours were included in the HR group.

Concerns regarding the short- and long-term cardiotoxicity caused by DOXO, as well as the belief that CDDP is probably the most effective drug for HB motivated the SIOPEL group to investigate whether SR-HB patients could be cured with a treatment strategy based on surgery and CDDP alone. For HR-HB patients, it was decided to intensify treatment by adding Carboplatin (CARBO) to the PLADO regimen used in the SIOPEL1 study, in a rapidly alternating administration sequence. Data indicating that Carboplatin was an "active" agent in HB were available at the time the study was launched

The relatively high proportion of SR-HB (70%) in the SIOPEL 1 study meant that it would be possible to compare the efficacy of a treatment strategy based on surgery and CDDP alone with that based on surgery and PLADO in a prospective randomised fashion. However, before launching this trial, a pilot study was deemed essential: i) to substantiate existing data on the efficacy of CDDP monotherapy, ii) to alleviate concerns about treating this malignancy with just one agent and iii) to investigate the toxicity of the proposed rapid (every 15 days) schedule of administering CDDP. By contrast, the HR-HB cohort of patients is smaller and prospective controlled trials more difficult to mount. In summary, the SIOPEL 2 was a pilot trail whose principle aims were to investigate efficacy and toxicity of two new therapeutic strategies: i) one based on CDDP monotherapy and surgery directed to a selected cohort of HBs with favorable presenting features and ii) an intensified multi-agent regimen directed to a group of HB with unfavorable clinical characteristics. The trial also provided data with which to examine the question of whether the notion of clustering patients into 2 risk groups was valid and helpful.

Response and resection rates for the entire SR-HB and HR-HB group were 90% (95%CI = 80-96%) and 78% (95%CI: 65-87%), and 97% (95%CI 87-99%) and 67% (95%CI 54-79%) including liver transplantation, respectively. For the two groups, the 3-year OS and progression-free survivals were 91% (±7%) and 89% (±7%), and 53% (±13%) and 48% (±13%) respectively. The short term toxicity of these regimens was acceptable, with no toxic deaths. Thus, the treatment strategy for SR HB based on CDDP monotherapy and surgery seems to be effective. Despite CT intensification, only half of HR-HB patients are long term survivors. Furthermore the SIOPEL2 trial supported the findings derived from the previous trial regarding the existence, within the HB population, of at least two risk groups for treatment failure, conventionally called SR and HR group. The difference in the 3-year OS and EFS observed between these two prognostic groups is striking.

The efficacy of CDDP monotherapy versus the historical combination CDDP/DOXO is now being compared by our Group in a prospective randomized trial (SIOPEL 3) in SR-HB while for HR HB an innovative regimen based on an intense use of CDDP will be soon prospectively evaluated

Other study reports on "high risk" HB

To some extent PRETEXT IV HB represent a comparable group of tumours to those HB that in the North American and German Co-operative Liver Tumour Trials, based on primary surgery, are considered in Stage III, meaning unresectable or tumour for which macroscopical residual disease was left after an initial attempt at surgical resection. Thus, some comparisons between different experiences, using modern series all based on platinum derived drugs, in this sub-set of HB can be attempted. The most relevant results achieved for stage III and metastatic HB patients in trials are reported in the Table1 and 2.

Table 1: Treatment results achieved in Stage III hepatoblastoma in trials adopting a primary surgery strategy

	Response Rate	Complete resection rate	Survival
CCG-823GF (24)			Estimated survival ~ 60%
POG 8697 (15)	30/31	24/31	4-years EFS% 67± 10.8%
INT-0089 (23)	Not reported	40/83	5-year survival 69% (SD=5%) 5-years EFS 64% (SD=5%)
POG 9345 (20)	19/22	15/22	5-years EFS 59%± 11 5-years OS 73% ± 10%
HB-94 (17)			DFS 76% (19/25) Median survival time of the entire population 58 months (range 32-93)

Table 2: Treatment results achieved in metastatic hepatoblastoma

	Response Rate	Complete resection rate	Survival
CCG-823GF (24)			Estimated survival ~ 25-30%*
POG 8697 (15)	6/8	1/8	4-years EFS% 12± 11.7%
INT-0089 (23)	Not reported	7/40	5-year survival 37% (SD=8%) 5-years EFS 25% (SD=7%)
POG 9345 (20)	8/11	4/11	5-years EFS 27%± 16 5-years OS 27% ± 16%
HB-94 (17)			DFS 21% (3/14) Median survival time of the entire population 58 months (r.32-93)
SIOPEL1 (27)	–	–	**5-years EFS 28% (95%CI 12-44)** 5-years OS 57% (95%CI 39-75%)
SIOPEL2 (42)	72%	60%	**3-years OS 44%**

Different strategies have been recently adopted to improve the survival of unresectable or metastatic HBs.

The POG group in North America adopted a novel treatment strategy based on intensification of pre-operative chemotherapy determined by tumour response, in order to improve the overall survival and decrease long term sequelae (POG 9345). Therapy was started with Carboplatin, followed by Carboplatin/VCR/5-FU and also by high dose CDDP and VP16 only for those patients whose tumours were not resectable after the fourth course of chemotherapy with Carboplatin/VCR/5-FU or who experienced no response (NR), or who had progressive disease (PD) at any time after course 2. Therapy consisted of high dose CDDP (40 mg/m^2/d) over 1 hour, followed by IV hydration on days 1 to 5, and Etoposide (100 mg/m^2/d and 3.3 mg/kg/d for patients < 10 kg) over 1 hour on days 2 to 4 immediately before HDDP for a total of two courses.

Twenty-four patients (80%) had at least a PR to Carboplatin/VCR/5-FU and 10 of those (71%) had a complete remission with chemotherapy and surgery; the 5 year EFS in this group of children is 71% ± 14%. Twelve patients were than treated with HD-Cisplatin/VP16 . Nine (75%) of 12 assessable patients (six stage III and three stage IV) had responses to HDDP-ETOP, with two patients experiencing PD. Each of the five patients who had a CR after HDDP-ETOP and resection has remained free of disease, with a minimum follow-up period of at least 5.5 years. Among the 12 patients who received HDDP-ETOP, 5-year EFS was 42% ± 14%. The 5-year event-free survival (EFS) for the entire cohort was 48% ± 9% with a 5-year overall survival of 57% ± 9% and a median follow-up period of 6.2 years for the surviving patients (range, 1.3 to 7.0 years). Superior survival was observed in patients without metastatic disease, with 5-year EFS of 59% ± 11% and 27% ± 16% in stage III and IV patients, respectively (P = .037). Overall estimates of survival at 5 years were similar, with 73% ± 10% in stage III and 27% ± 16% in stage IV, respectively (P = .012).

The treatment results of the POG trial, considering the entire cohort of patients and the two subgroups of children included (the unresectable and the metastatic ones, assuming similarities between the POG Stage III HB and the SIOPEL PRETEXT IV tumours) seem to be almost superimposable to ones achieved in the SIOPEL studies (SIOPEL HR group: 3-year EFS 48% (±13%), 3-year OS 53% (±7%); POG data on unresectable/metastatic HB: 5-year EFS 48% ± 9%; 5-years OS 57% ± 9%; for the different subgroups see Tables 6.2, 6.3 & 6.4)

The German group adopted a similar strategy to the one used by the POG group, based also on primary surgery. All children started with Ifosfamide/Cisplatin/Doxorubicin and then the ones not achieving a response to the first 2 cycles and/or with metastatic and advanced disease switched to Carboplatin/VP16 (17). In their report responses by stage are not available. It is only said that 85% children responded after the first two courses of chemotherapy and that 18 children ended-up being treated with CARBO/VP16. Twelve children responded, and, in one child with stage IV HB, complete disappearance of lung metastases was registered. Six patients were non-responders: Five of them had an undifferentiated or embryonal HB with anaplastic components. All children who did not have a tumour response died (p= 0.0013). There was a minor difference in response to Carboplatin/VP16 in patients who had metastatic HB compared with patients who had advanced HB. The DFS of patients with stage III HB and of the ones presenting with metastases are 76% (19/25) and 21% (3/14) respectively with a median survival time of the entire population of 58 months (range 32-93). Based on this experience, the German group is now recommending high dose VP16/Carboplatin chemotherapy with stem cell rescue for all children with stage III HB, with vascular invasion and/or multifocal, spreading tumour and stage IV HB.

North American investigators tried to intensify therapy by increasing the dose intensity of platinum derived agents, alternating the use of Carboplatin (700 mg/m^2) and Cisplatin (100mg/m^2) every 14 days (25). It appears that despite the initial promising results of the pilot trial (2 CR, 8 PR out of the first 13 patients with 9 patients alive with no evidence of disease for greater than 21 months) this regimen when tested prospectively versus the Carboplatin/VCR/5-FU brought such unsatisfactory results that the trial was prematurely closed (M. Malogolowkin, personal information).

Also for HB it is predicted that a better understanding of the intimate biologic characteristics dictating tumour cell growth will allow a more significant improvement of these present treatment strategies for childhood HB

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Treatment of Hepatoblastoma - Role of surgery

Jean Bernard Otte

Whatever the histology of a malignant liver tumuor, radical resection is a prerequisite for cure.

Modern treatment of malignant liver tumours is multidisciplinary. Preoperative chemotherapy has been validated as a cornerstone for hepatoblastoma. Surgery should be scheduled in close concertation with oncologists. Moreover, these children should be enrolled in a prospective, well controlled trial.

The surgeon should have expertise in any type of liver resection, including the most extended ones, like trisegmentectomies, either left or right or resection of centrally located tumours. He must also have access to and experience with modern tools needed in liver surgery, like the intraoperative ultrasonography, argon beam coagulator, ultrasonic dissector, blood saver and porto-cavo-jugular veno-venous bypass. Collaboration with an unit of paediatric intensive care is mandatory as well with a lab of pathology for frozen sections.

Personal experience of the liver surgeon with all variant techniques and follow-up of liver transplants is optimal . These requirements mean that children with major liver tumours should be treated in expert centres, hopefully with significant experience in liver transplantation.

Indications for liver transplantation (LTX) - Hepatoblastoma

When HB is unresectable with a partial, even extended hepatectomy, the best

and only reasonable option is a total hepatectomy with a liver transplant (J.B. Otte, 2004). In order to prevent the development of tumor resistance to chemotherapy and re-growth, transplantation should not be delayed and should be synchronized with chemotherapy as is conventional surgery. Due to the usual time constraints with deceased donor organs, and despite the use of split liver grafts, a suitable donor organ may not become available and living-related donation is a valuable option.Living donation offers the possibility of the best timing in regard with preoperative chemotherapy.

Unresectable HB are those extending to the four liver sections (PRETEXT IV) (Dall'Igna et 2003) and those, either PRETEXT III or II , with close proximity with or extension into the major venous structures (portal vein or hepatic veins), incurring the risk of non radical resection if a partial hepatectomy is performed (Srinivasan et 2002) Active metastatic disease or extrahepatic deposits after chemotherapy are, of course, contraindications to transplantation. However, LTX remains an acceptable option, with good long term results, for children who present with lung metastases which subsequently clear during chemotherapy (Pritchard 2000).Thoracotomy may be indicated for surgical excision of residual disease if any doubt subsists (Perilongo 2000, Schnater 2005). Macroscopic venous invasion (portal or hepatic veins) is also not an absolute contraindication to transplantation as long as radical excision is possible (G. Reyes 2000; J.B. Otte 2004)

There is no evidence that transplant patients should be treated differently from those undergoing radical resection with regard to post-surgery chemotherapy. The arguments to give chemotherapy after partial hepatectomy hold true for total hepatectomy and LTX, at least for extrahepatic micrometastases. Increased toxicity from combining chemotherapy with tacrolimus-based immunosuppression has not been observed if a reduced level of immunosuppression is maintained with low trough levels of tacrolimus (Otte 2005).

Disease-free, long-term patient survival after primary transplantation is about 80%, in contrast with much lower survival rate, around 30 %, in case of rescue transplantation for incomplete tumour resection or relapse after partial hepatectomy (Otte 2004).

Hepatocellular carcinoma

Experience with LTX in children with HCC is very limited; guidelines on indications are mainly derived from experience gained with adult patients (Mazzaferro 1996). The presence of multifocal tumour or underlying liver disease may preclude partial hepatectomy. Patients with an unresectable HCC, either because of intrahepatic extent or underlying cirrhosis, should be assessed for transplant. The Milan criteria have been used to define adults with cirrhosis-associated HCC who are suitable for LTX: no more than 3 tumours, each no more than 3 cm in size, or a single tumour not more than 5 cm in diameter. When a single tumor is present in an otherwise normal liver, the present cut-off of 5 cm in diameter might be expanded to 6.5 (Yao 2001) or even 7 cm (Roayaie 2002) without compromising survival. In any case, macroscopic venous invasion and extrahepatic extension, including lymph node spread and distant metastases, remain absolute contraindications to LTX.

Epithelioid hemangioendothelioma

Total hepatectomy and LTX for this type of tumor is reported in adults, with a 5-year, disease-free survival of 60% (Madriaga 1995). However, reports of LTX in children with this tumour are anecdotical and several centres have had bad experience with rapid recurrence and death (Sharif 2004; Otte et al 2005)

PLUTO

The SIOPEL strategy group has decided to create an international registry called PLUTO, which stands for *P*ediatric *L*iver *U*nresectable *T*umor *O*bservatory for online registration of children undergoing a liver transplant, either primarily or as a rescue for incomplete partial liver resection or relapse after partial liver resection.All patients enrolled in the American COG feasibility study who undergo a transplantation will be enrolled into PLUTO as an integral part of the COG study.

The aim is to establish a multicenter, international database with propective registration of pediatric (<18 years) patients presenting with unresectable tumor (hepatoblastoma, hepatocellular carcinoma, epithelioid hemangioendothelioma) undergoing a primary or a rescue transplantation.A remote date entry system has been created which is accessible online, free of charge for contributing centers.

Paediatric oncologists, hepatologists and pediatric /transplany centers are encouraged to visit the website: http://pluto.cineca.org for registration of their patients (Otte 2006).

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Myelodysplastic syndrome (MDS) and Juvenile Myelomonocytic Leukemia (JMML)

Henrik Hasle

Classification - The FAB and WHO classifications

The French-American-British (FAB) cooperative group produced the first systematic attempt of a classification dividing myelodysplastic syndrome (MDS) into five subgroups: refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess of blasts (RAEB), RAEB in transformation (RAEB-t), and chronic myelomonocytic leukemia (CMML)[1;2]. Distinction among the subtypes was based on the proportion of blasts in the peripheral blood (PB) and bone marrow (BM) and the degree of monocytosis in the blood. The WHO classification of hematological malignancies incorporates clonal cytogenetic abnormalities and lowered the threshold for distinguishing acute myeloid leukemia (AML) from MDS from 30% to 20% of blasts in the BM [3].

Both the FAB and the WHO proposals were based on review of adult cases although the WHO classification recognizes juvenile myelomonocytic leukemia (JMML) as a separate entity. There are many differences between MDS in children and adults, e.g. RARS is exceedingly rare in children, and constitutional abnormalities are observed in a large fraction of children but very uncommon in adults. The 5q- syndrome is considered a unique entity in adults but has not been reported in children.

Current approach to the classification of childhood MDS

Internationally consensus has been achieved on the classification of MDS in childhood[4]. Myelodysplastic and myeloproliferative disorders in children are separated into three main groups; MDS, JMML, and Down syndrome disease **(Table 1)**.

Table 1: Diagnostic categories of myelodysplastic and myeloproliferative diseases in children

I. Myelodysplastic / Myeloproliferative Disease

- Juvenile myelomonocytic leukemia (JMML)

II. Down Syndrome (DS) Disease

- Transient abnormal myelopoiesis (TAM)
- Myeloid leukemia of DS

III. Myelodysplastic Syndrome (MDS)

- Refractory cytopenia (RC) (PB blasts <2% and BM blasts <5%)
- Refractory anemia with excess blasts (RAEB) (PB blasts 2-19% or BM blasts 5-19%)
- RAEB in transformation (RAEB-t) (PB or BM blasts 20-29%)

MDS is subdivided into refractory cytopenia (RC), RAEB and RAEB-t. The classification is used for both de novo and secondary MDS. The change in nomenclature from RA to RC reflects that anemia is not a prerequisite for the diagnosis but only seen in about 50% and less frequent than neutropenia, thrombocytopenia and macrocytosis[5]. It is suggested to retain the RAEB-t entity but to emphasize that the blast count is insufficient to differentiate AML from MDS.

Myeloid leukemia in children with Down syndrome has unique features and is kept separate as a distinct entity (please see below).

The Toronto group has published a descriptive system designed to assess children with MDS according to category, cytology and cytogenetics (CCC)[6]. The CCC system emphasizes the important characteristics of each patient but has an infinite number of possible

subgroups making it difficult to use in clinical practice or research.

The new pediatric modification of the classification[4] emphasizes the clinical relevant subtypes of pediatric MDS and eliminates adult subtypes that are rare or unseen. However, we will still face borderline cases difficult to fit into the classification.

Primary and secondary MDS

MDS can arise in a previously healthy child and is conformingly named "de novo" or "primary". It may also develop in a child with a known predisposing condition and referred to as "secondary". Secondary MDS is seen in patients a) after chemo- or radiation therapy (therapy-related MDS), b) with inherited BM failure disorders, c) with acquired aplastic anemia and d) with familial MDS. It is to be recognized, however, that children with so-called "primary" MDS may have an underlying yet unknown genetic defect predisposing them to MDS at young age. Therefore, the distinction between primary and secondary disease may become arbitrary.

Myeloid neoplasias in patients with predisposing conditions share the biological characteristics of MDS regardless of the presenting blast count. The prognosis appears to depend primarily on the cytogenetic profile.

Myeloid leukemia of Down syndrome

Individuals with Down syndrome (DS) have a more than 50-fold increased risk of leukemia during the first five years of life[7], even after excluding the transient myeloproliferative disorder (TMD) indistinguishable from leukemia occurring in 10 % of newborns with DS. About half the leukemias in children with DS are myeloid often presenting with features of MDS.

Myeloid leukemia in children with DS occurs characteristically at 1-4 years of age with an excess of megakaryoblasts, and almost uniform presence of *GATA1* mutation[8]. In contrast to TMD, myeloid leukemia in older children is fatal if untreated but responds to AML treatment with a very favorable prognosis[9;10]. The myeloid leukemia seen in young children with DS is unique and classified under the unifying term *myeloid leukemia of DS* (ML-DS)[4]. ML-DS is preferred to acute megakaryoblastic leukemia because other phenotypes are observed sharing the same biologic and clinical characteristics. It is no longer appropriate to use the terms MDS and AML in young children with DS.

Epidemiology

MDS and JMML are both uncommon in children and adolescents, each constituting less than 5% of all hematological malignancies. Using the pediatric WHO classification the annual incidence per million children 0-14 years of age is 1.8 of MDS, 1.2 of JMML, and 0.9 of myeloid leukemia of Down syndrome[4]. There is an equal sex distribution in MDS and a median age at presentation of 6.8 years[11-14]. JMML shows in contrast a very low age at onset (1.8 years) and a male predominance. Constitutional abnormalities are relatively frequent in both MDS and JMML. The most common abnormalities are listed in **Table 2**.

Table 2: Abnormalities associated with JMML and MDS in children

A.	associated with JMML
Constitutional conditions	
	Neurofibromatosis type 1 (NF1)
	Noonan syndrome
	Trisomy 8 mosaicism
B.	associated with MDS
	Constitutional conditions
	Congenital bone marrow failure
	Fanconi anemia
	Kostmann syndrome
	Shwachman-Diamond syndrome
	Blackfan-Diamond anemia
	Trisomy 8 mosaicism
	Familial MDS (at least one first degree relative with MDS/AML)
Acquired conditions	
	Prior chemotherapy/radiation
	Aplastic anemia

Diagnostics - MDS

The two major diagnostic challenges are to distinguish MDS with a low blast count from aplastic anemia (AA) and other non-clonal BM disorders, and to differentiate MDS with excess

of blasts from AML. The traditional classification has been based on morphology but a number of additional factors need to be considered.

Refractory cytopenia

Myelodysplasia may occur in the BM in a variety of disorders of very different etiologies, e.g. infection[16], drug therapy[17] and chronic disease[18]. Non-clonal disorders with dysplastic features, e.g. mitochondrial disorders like Pearson syndrome, should not be considered as MDS.

It may be difficult to diagnose MDS in children who have a low blast cell count and no clonal marker. The proposed minimal diagnostic criteria[4] may be helpful in this situation **(Table 3)**.

Table 3: Minimal diagnostic criteria for MDS

At least 2 of the following:

- Sustained unexplained cytopenia (neutropenia, thrombocytopenia, or anemia)
- At least bilineage morphologic myelodysplasia
- Acquired clonal cytogenetic abnormality in hematopoietic cells
- Increased blasts (>5%)

Since hematopoiesis is often dysplastic in patients with congenital BM failure disorders, it is suggested diagnosing MDS in these patients only if the BM blast count is increased, a persistent clonal chromosomal abnormality is present or hypercellularity in the BM develops in the presence of persistent PB cytopenia.

RARS is extremely rare in children. The finding of sideroblastic anemia should prompt investigation for possible mitochondrial cytopathy or disorders of heme synthesis[19].

A trephine biopsy of good quality is mandatory in the evaluation of a child with suspected AA or MDS. A careful search for morphological characteristics at diagnosis will often establish a distinction between the two entities[20]. Hypoplastic MDS tends to show sparsely scattered granulopoietic cells, patchy islands of immature erythropoiesis and in most cases decreased megakaryopoiesis and in some micromegakaryocytes. Overexpression of p53 is suggestive of MDS[21].

Refractory anemia with excess of blasts (RAEB) and RAEB in transformation (RAEB-T)

RAEB is defined by a BM blast count between 5 and 20%. Auer rods are no longer a discriminator for classification. Patients with recurrent cytogenetic abnormalities typically associated with AML, e.g. t(15;17) (PML/RARalfa), t(8;21) (RUNX1/CBFA2T1), inv(16) (CBFB/MYH11), t(9;11) (MLL/MLLT3), should be diagnosed and treated as AML regardless of the blast count[22].

MDS and true de novo (TDN)-AML display significant differences in pathogenesis and natural course[23]. The cytogenetic differences predicting response to therapy in MDS/AML may reflect the underlying biological nature of the disease. TDN-AML is a chemo-sensitive disease characterized by specific recurring translocations, whereas MDS and secondary AML is characterized by numerical chromosomal abnormalities and are typically resistant to chemotherapy. Patients with adverse cytogenetics have a poor response to therapy irrespective of the proportion of blasts in the BM and have been described as MDS-related AML (MDR-AML)

Monosomy 7 is the most common acquired abnormality in children with MDS[11;14]. Children with monosomy 7 and MDS have an outcome similar to MDS patients without monosomy 7, whereas patients diagnosed as AML with monosomy 7 have a lower response rate to chemotherapy and a higher relapse rate compared with AML without –7[24;25]. Monosomy 7 may be regarded as a marker of an MDS-like disease.

The WHO classification[3] suggested abolition of the category of RAEB-t including most of these patients as AML with multilineage dysplasia. The cut off point for diagnosis of AML was lowered from the traditional 30% to 20% blast cells. This distinction is clearly an arbitrary one and there must in practice be a continuum between RAEB and AML. There are no data to indicate whether a 20% blast cell cut off is useful in pediatrics. A British study suggested a better outcome following AML therapy in patients with RAEB-t compared with RAEB[26], however, this was not found in an American study[25]. Until more data are available it is suggested maintaining the RAEB-t category in children.

It is important to recognize that any threshold of blast percentage to separate MDS from AML is

a surrogate marker for the underlying biological behavior of the disease. In patients with ambiguous blast count a more clinical relevant approach may be based upon clinical features, cytogenetics and serial assessment of the BM rather than predicting clinical behavior from a single examination.

JMML

JMML is a unique pediatric disorder previously referred to as JCML or CMML. Diagnostic criteria for JMML are listed in **Table 4**[27]. Blood film appearance is characteristic and often more helpful in diagnostics than BM smear. Mutations in the *Ras* gene is seen in 20%, in *PTPN11* 35%, *NF1* gene in 15% and clinical NF1 in another 15%, molecular genetics has therefore become very helpful in diagnosing JMML[28]. JMML includes patients with monosomy 7 previously considered to represent a distinct hematological disorder described as the monosomy 7 syndrome. There are no major clinical differences between JMML in children with and without monosomy 7[29 24].

Natural course and prognostic factors in MDS

Children with RC or low grade RAEB may show a long and stable clinical course without treatment. Blood transfusions are only required infrequently and severe infections are rarely seen. The condition may smolder with unchanged cytopenia for months or even years but will eventually progress in most patients. In a series of 67 children with primary RC, four died from complications of pancytopenia prior to therapy or progression and 20 progressed to more advanced MDS at a median of 1.7 years from presentation[5]. Although RC with monosomy 7 is associated with a higher risk of progression both RC and RAEB patients with monosomy 7 may show stable disease without treatment for several years[24]. Once progression has occurred the outcome is inferior even after SCT[5;30]. Spontaneous regression of MDS has occasionally been reported in the literature[31].

The International Prognostic Scoring System (IPSS) for MDS weighted data on BM blasts count, cytopenia and cytogenetics and separated patients into four prognostic groups[32]. The IPSS has been very useful in adults but is less informative in children[33].

Treatment of MDS

MDS is a clonal early stem cell disorder with very limited residual non-clonal stem cells. Myeloablative therapy is therefore the only treatment option with a realistic curative potential. A diversity of therapy strategies like hematopoietic growth factors, differentiating agents, hormones, amifostine, low dose cytotoxic drugs, or experimental agents have been investigated in adults and in the elderly not candidates for SCT. None of these approaches have been documented to prolong survival and

Table 4: Diagnostic guidelines for JMML adopted from [27]

Suggestive clinical features	Hepatosplenomegaly
	Lymphadenopathy
	Pallor
	Skin rash
Laboratorial criteria	No Ph chromosome, no bcr-abl rearrangement
Minimal criteria	PB monocyte count > 1 x 10^9/L
(all 3 must be fulfilled)	Bone marrow blast count < 20%
Criteria for definite diagnosis	Hemoglobin F increased for age
(at least 2 must be fulfilled)	Myeloid precursors in blood smear
	White cell count > 10 x 10^9/L
	Clonal abnormality
	GM-CSF hypersensitivity of myeloid progenitors

they are generally not indicated in children and adolescents. Given the lack of recurrent molecular abnormalities in MDS rational drug development aiming at molecular targeted therapy is problematic.

Immunosuppressive therapy has been successful in some adults with MDS and low blast count, especially in patients with BM hypoplasia and HLA-DR15 (DR2)[34]. Other studies have been less optimistic reporting a significant burden of side effects[35]. Preliminary data have shown a few long lasting responses in children with RC treated with anti-thymocyte globulin[36]. Whether immunosuppressive therapy can result in sustained responses in childhood RC is not known.

Children with MDS are at high-risk of cytopenia related complications and optimal supportive care should be the primary focus during all phases of the disease course.

Conventional intensive chemotherapy without SCT is unlikely to eradicate the primitive pluripotent cells involved in MDS rendering the therapy non-curative in most patients. Most studies found a significant morbidity and mortality of induction chemotherapy with a complete remission rate of less than 60%, many relapses, and overall survival less than 30%[13;25]. The treatment related mortality has been between 10 and 30%[25;26]. A few studies have reported an outcome in MDS patients not significantly different from that in AML[26;37]. Some studies suggested that those with RAEB-T or AML following MDS have a superior outcome compared with RAEB [25;26] indicating that RAEB-T consists of a heterogeneous group of patients and that a purely morphologically based classification is insufficient for a treatment relevant stratification[4].

SCT is the therapy of choice for virtually all forms of MDS in childhood. Studies specifically addressing the question of SCT in children have indicated a probability of disease-free survival (DFS) following transplant with an HLA-matched family donor (MFD) of about 50%[38;39]. Children receiving a graft from an HLA-matched unrelated donor (MUD) have previously suffered a higher transplant-related mortality (TRM) and lower DFS but more recent studies have shown survival following MUD-SCT comparable to MFD-SCT[40;41]. A preparative regimen consisting of busulfan, cyclophosphamide and melphalan[42] has shown a high anti-leukemia effect.

For patients with advanced MDS the potential benefit of AML-type induction chemotherapy prior to SCT to reduce relapse and improve DFS remains a controversial issue. Prior chemotherapy may increase TRM[43]. Considering the significant morbidity and mortality of induction chemotherapy and the high rate of TRM following SCT most children with MDS may benefit from SCT as first line therapy sparing the toxicity related to induction chemotherapy. Children without a matched donor and progressive disease should be considered for haploidentical SCT[44].

Relapse following SCT is associated with a very grave outcome. Especially early relapse detected by increasing mixed chimerism may benefit from withdrawal of immunosuppressive therapy and donor leukocyte infusion[45].

Children with MDS secondary to chemo- or radiation therapy generally have a very poor survival. AML-type therapy may induce remission but very few patients remain in remission and even SCT has been reported to offer cure to only 20 - 30% of patients[46;47].

The few published cases of SCT in MDS arising from congenital BM failure disorders or acquired aplastic anemia indicate a poor outcome for this heterogeneous group of patients. Early SCT before neoplastic transformation or during less advanced MDS may be associated with improved survival[48;49].

Natural course and prognostic factors of JMML

JMML is a rapidly fatal disorder if left untreated. Low platelet count, age above 2 years, high Hemoglobin F and high bone marrow blast count at diagnosis are the main factors predicting a short survival[29]. Non-transplanted children presenting with a low platelet count ($< 33 \times 10^9$/L) died within a year from diagnosis[29]. Blastic transformation is infrequent with JMML and most untreated patients die from organ failure due to infiltration of the leukemic cells.

Treatment of JMML

Clinical and hematological responses in JMML have most consistently been described for 6-mercaptopurine administered as single-agent[50]. There are, however, no data indicating that

therapy with 6-mercaptopurine influences the length of survival.

Intensive chemotherapy is mostly unsuccessful in JMML because of an increased risk of treatment related death, a low rate of true remissions and long-term survival less than 10% [29;50;51]. Allogeneic SCT is the only curative approach for JMML resulting in long-term survival in more than half the patients [52-54]. If no family donor is available a matched unrelated donor SCT is recommended. Generally, SCT shortly after diagnosis is advocated, and younger age at SCT may predict for improved survival. A conditioning regimen of total body irradiation (TBI) and cyclophosphamide has often been used[55]. Radiation-induced late effects like endocrine dysfunction including severe growth retardation and neuropsychologic sequalae may be especially deleterious for this group of very young children. Therefore, avoiding TBI is particularly attractive in JMML. Several investigators have reported similar outcome for patients conditioned with TBI compared to non-TBI regimens[52;53]. In a retrospective analysis of the European Working Group on MDS in Childhood (EWOG-MDS) busulfan-based myeloablative therapy offered a greater anti-leukemic efficacy than TBI[54]. The current study of EWOG-MDS uses a preparative regimen with busulfan, cyclophosphamide and melphalan has produced event-free survival around 50% with no difference between related and unrelated donor[54]. Disease recurrence remains the major cause of treatment failure. Too intensive GvHD prophylaxis increases the risk of relapse[54] whereas acute or chronic GvHD is associated with a lower risk of relapse[52-54]. Relapse occurs early at a median of 2-4 months from transplantation[53;54] and generally within the first year. Re-emerging donor cells and frank relapse have been successfully eradicated by reduction of ongoing immunosuppressive therapy[56]. Reducing intensity and duration of GvHD prophylaxis may significantly contribute to successful leukemia control. Donor lymphocyte infusion (DLI) in JMML relapse is largely unsuccessful[57].

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