

37<sup>th</sup> Congress of the International Society of Paediatric Oncology  
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## EDUCATION BOOK

# International Society of Paediatric Oncology





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Malignancies – the role of Biologicals

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## **A c k n o w l e d g e m e n t s**

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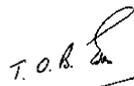
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## P r e f a c e

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



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— SECTION **A** —

# Machinery of the Cell - Principles of targeted diagnoses and therapies

Elizabeth R. Lawlor, MD, PhD

**A**s demonstrated by the success of imatinib (Gleevec, STI-571) therapy for chronic myelogenous leukemia (CML) and the recent emergence of several tumour-directed therapeutic antibodies, the age of biologically targeted therapies for cancer is upon us. The field of paediatric oncology has already been impacted and, in the future, will be radically altered by these new approaches. It is imperative that treating physicians understand the fundamental concepts behind the development and utility of these agents.

Traditional anti-cancer drugs have relied upon irreparably disrupting DNA synthesis in and division of cycling cells. Unfortunately, this approach is neither particularly efficient nor specific as not all cancer cells proliferate rapidly (e.g. our emerging knowledge of cancer stem cells) and it is very toxic to many normal cells that rely on proliferation for homeostasis and wound repair (e.g. hematopoietic progenitor and mucosal epithelial cells). The newer classes of biologic agents are designed to target processes that are more specific to either the malignant cell itself or to its surrounding environment such that selective tumour killing is possible. This increased specificity has the combined benefit of improving clinical and biologic remission whilst decreasing treatment-related morbidity and mortality.

In this talk I will review recent developments in our understanding of normal and cancer cell biology and how they have led and are leading to the development of novel biologically targeted therapies. Beginning with the cell membrane and cell surface receptors, I will review how normal cells receive and process signals from their external environment and transmit these signals to the nucleus where orchestration of an appropriate response is initiated. An inappropriate signal or response can facilitate

malignant transformation by disrupting normal cellular processes such as cellular proliferation, apoptosis (programmed cell death) and differentiation. We now recognize that the cellular machinery controlling these processes is frequently deranged in human cancer.

The cell membrane is populated by numerous receptors of multiple different types, including the protein tyrosine kinase receptor family (RTK). These receptors bind to ligands present in the extracellular matrix or on the surface of other cells and thus initiate complex intracellular signaling cascades that transmit messages to the nucleus. Genetic mutations that lead to over-expression or ligand-independent activation of RTKs are seen frequently in cancer. Such constitutive activation of RTK signaling can cause unchecked proliferation and resistance to apoptosis, thus contributing to the cancer phenotype. Recently, antibodies have been designed that specifically target these receptors and inactivate them. Examples of RTKs frequently mutated in cancer include EGF-R (ErbB1) and Her2/Neu (ErbB2). Receptor-specific monoclonal antibodies, such as gefitinib (Iressa) and cetuximab (Erbix), directed at these targets have already demonstrated efficacy in clinical trials and are being widely used in the treatment of many different tumour types. Antibodies and other agents designed to target cell surface receptors will feature more and more prominently in the future.

Once a signal has been received by the receptor at the cell membrane, the signal is transmitted to the nucleus. Two of the key signaling pathways that are important to both normal and cancer cell biology are the Ras-Raf-MAPK and the PI3K-AKT pathways. Transmission of a signal through these pathways occurs via complex networks of lipids and proteins that interact to activate and de-activate one another in sequence and in

parallel. In most cases, activation of a substrate is achieved by phosphorylation of specific amino-acid residues (tyrosine, serine or threonine) by enzymes called kinases, whereas inactivation most commonly involves dephosphorylation by phosphatases. Mutations in protein kinases are frequent occurrences in cancer and they uniformly lead to constitutive activation of the kinase (e.g. Raf mutations, Bcr-Abl). Likewise, loss of the lipid phosphatase gene *PTEN* results in constitutive activation of the PI3K-AKT pathway and enhanced cell survival. Imatinib (Gleevec) was designed to specifically inactivate the kinase function of the Bcr-Abl fusion protein and is the prototype example of a biologically targeted small molecule. The development of small molecules directed at inactivating protein kinases constitutes one of the most active and promising areas of research in targeted therapeutics. Unfortunately, acquisition of drug resistance is an emerging problem that will also need to be tackled in parallel.

Once a signal traverses the cytoplasm and is received by the nucleus, gene transcription is initiated. In many cases extra-cellular signals lead to the induction of genes that control cell cycle entry and proliferation such as *c-Myc* and *cyclin D1 (Ccd1)*. Deregulation of the cell cycle and inappropriate proliferation are among the hallmarks of cancer and they are often a result of aberrant transcription factor activity. Many paediatric malignancies are characterized by genetic mutations that result in deregulation of physiologic transcription factors (e.g. *c-Myc*, *N-Myc*) or in the expression of novel proteins that either function as transcription factors themselves or serve to disrupt normal transcription (e.g. *Pax3/7-FKHR*, *EWS-FLI1*, *TEL-AML1*). Although traditionally more difficult to target, nuclear proteins and transcription factors are gaining momentum as potential therapeutic targets. This is largely because of the development of novel technologies such as RNA interference and improved delivery methods involving nanotechnologies. Targeted therapy of deregulated transcription factors is certain to expand in the future.

Resistance to apoptosis is another hallmark of the cancer cell and restoration of normal apoptosis pathways is a focus of current

research efforts. Apoptosis results as a consequence of activation of either extrinsic or intrinsic signaling pathways. The intrinsic pathway is initiated in response to a DNA damage signal and centers around the mitochondria and the Bcl2 family of proteins. The extrinsic pathway is initiated by ligand binding of death-receptors at the cell membrane and can also involve members of the Bcl2 family. Over-expression of the anti-apoptotic protein Bcl2 is seen in many tumours and down-regulation of its expression by an anti-sense molecule (Genasense) has been used with variable success to restore normal apoptosis to cancer cells. More recently, small molecules which mimic the effect of Bcl2 family members that are pro-apoptotic (the BH3-only proteins) have been developed and are showing promise as anti-cancer agents in pre-clinical studies. Death receptors on the cell surface include members of the TNF-receptor super family, among them the receptors for TRAIL and FAS. When these receptors bind their respective ligands cell death pathways are activated and apoptosis results. Therefore, TRAIL and other death-receptor ligands have been developed as anti-cancer drugs. A limitation of TRAIL has been that successful propagation of the death signal requires adequate levels of caspase 8. In many cancers, including neuroblastoma, methylation of the caspase 8 promoter prevents its transcription and silences its expression. Such epigenetic silencing by promoter methylation occurs frequently in human cancer and leads to the down-regulation of expression of many critical cell-cycle regulatory genes. This silencing can therefore induce resistance to TRAIL and also abrogate intrinsic tumour suppressor mechanisms within the cell. Treatment of cancer cells with demethylating agents, such as 5-azacytidine, is now being evaluated as a means of restoring expression of silenced genes.

In addition to cell-intrinsic biology and signaling, it is crucial that the cancer cell be considered in the context of its environment if novel therapies are to be successful. Cancer cells are anchored to one another and to the extra-cellular matrix and they exist in an environment rich in macrophages and other inflammatory cells. All of these factors contribute to the mediation of cellular processes and, as such, can be exploited

as therapeutic targets. Moreover, the angiogenic potential and requirement of all tumours enables anti-angiogenic strategies. Current agents in early and late clinical trials have been designed to disrupt cell-matrix interactions (Cilengitide), to block the formation of new blood vessels (Bevacizumab/Avastin) and to harness an anti-tumour immune responses (patient-specific cytotoxic T-cells). The role of the surrounding environment in cancer cell proliferation and survival should not be underestimated and drugs which target the tumour in relation to its environmental milieu will be integral to future treatment protocols.

In summary, the cellular machinery that controls cell growth, proliferation, differentiation and

death is normally tightly regulated and deviations from the norm are quickly corrected in order to maintain homeostasis. Disruptions in this machinery, as a result of genetic mutation, are responsible for the genesis and maintenance of human tumours. Advances in our understanding of normal cellular biology and how it is disrupted in cancer have enabled the development of novel, biologically targeted therapies that exploit the unique cell-intrinsic and cell-extrinsic characteristics of a tumour. This targeted approach to cancer therapy is already a reality and will be the foundation of treatment regimens of the future.

# Making the Correct Diagnosis—Nuclear Medicine Tools

Helen Nadel

**N**uclear Medicine examinations are integral in the management of children with cancer. Imaging with conventional gamma camera systems, hybrid gamma camera systems and radiopharmaceuticals; and with positron emission tomography (PET) and PET/CT systems and positron emitters can be used for staging, metastatic work-up, surveillance follow-up, therapeutic response assessment and treatment. Tumors that are routinely evaluated include, osteogenic sarcoma, Ewing's sarcoma, rhabdomyosarcoma, neuroblastoma and lymphoma. Leukemia, brain tumors and thyroid tumors, renal and hepatic tumors may also be imaged using scintigraphic techniques.

The use of image co-registration of functional studies obtained with gamma cameras or PET cameras with anatomic imaging on CT and MRI provide a further level of evidence of the significance and specificity of diagnostic findings. This can be accomplished with sequential acquisition of data in combined PET-CT or gamma-camera-CT machines or by co-registration digitally of individually acquired studies not temporally related. The clinical impact of these combined modalities are becoming established in adults <sup>(1-5)</sup>.

## Musculoskeletal Tumors

Primary malignant bone tumors such as osteogenic sarcoma, Ewing's sarcoma and rhabdomyosarcoma involving bone will appear as intense tracer uptake on skeletal scintigraphy with Tc-99m methylene diphosphonate (MDP) standard bone scans. MDP bone scintigraphy is still the appropriate way to survey the skeleton for extent of disease including metastatic or multifocal disease including skip lesions. Pulmonary metastases of osteosarcoma can show uptake of Tc-99m bone seeking radiopharmaceuticals, but the sensitivity for uptake is much less than the sensitivity for

metastatic disease detected with CT scan.

Non-metastatic increased Tc-99m MDP activity may be found after amputation or limb salvage procedures and the various patterns need to be recognized as non-neoplastic. A pattern of non-specific increased activity in the axial skeleton and/or juxtaarticular areas on MDP bone scintigraphy can also be seen in patients who are given colony stimulating factors (CSF).

The gold standard for the assessment of histologic response in osteosarcoma after neoadjuvant chemotherapy has been the evaluation of tumor necrosis on the histologic specimen at the time of definitive surgery; either limb salvage or amputation. Thallium-201 and Tc-99m sestamibi have been used as "surrogate markers" for the non-invasive assessment of this histologic response in osteogenic sarcoma<sup>(6)</sup>. The most important factor in the use of scintigraphy for histologic response assessment and detection of residual tumor is the determination of baseline tumor avidity for the specific radiopharmaceutical. The determination of baseline tumor avidity is best performed at the time of initial staging for all suspected tumors in order to minimize the influence of tissue distortion and inflammation after biopsy or surgery. Histologic tumor response assessment relies on the finding of a decrease in thallium or sestamibi uptake between pre and post treatment scans to indicate good tumor histologic response. Poor tumor histologic response will show persistence of abnormal radiopharmaceutical uptake.

2-[fluorine-18]-fluoro-2-deoxy-D-glucose (FDG) PET may also be helpful in monitoring tumor histologic response. In tumors treated with chemotherapy, FDG-PET accumulation decreased more homogeneously throughout the tumor, in responsive cases. FDG-PET uptake has been shown to more accurately reflect viable

metabolically active tumor. PET may also detect metastatic foci, but occasionally nonspecific uptake not due to malignant disease may show increased uptake on a whole body PET scan. In a study of 18 patients with bone tumors Franzius et al showed on FDG PET imaging tumor to non-tumor ratios in all patients who had good responses ratios decreased more than 30% <sup>(7)</sup>. In patients with poor responses tumor to non-tumor ratios increased or showed less than 30% decrease in contrast to the good responders. Hawkins et al also found FDG-PET with quantitation to correlate with tumor histologic response after neoadjuvant chemotherapy in both osteosarcoma and Ewing's sarcoma <sup>(8)</sup>. Quantitation by response assessment of maximum standardized uptake values (SUVmax) before and after therapy on PET studies was evaluated in 45 patients. They found that patients with a baseline tumor SUVmax  $\geq 6$  and  $< 40\%$  decrease in FDG uptake were at 90% risk of systemic disease recurrence at 4 years from the time of initial diagnosis. Patients whose tumors had a  $\geq 40\%$  decline in the SUVmax in response to chemotherapy were at a significantly lower risk of recurrent disease and death after complete resection and adjuvant radiotherapy <sup>(9)</sup>. FDG PET is also useful in combination with MR imaging to help distinguish viable tumor from post therapeutic changes in patients with bone and soft-tissue sarcomas <sup>(10)</sup>.

MDP bone scintigraphy in Ewing's sarcoma is important in initial staging of the tumor and for following patients after therapy. Scintigraphy at presentation will commonly show intense uptake of radiopharmaceutical in the lesion. Ewing's sarcoma may show metastases at diagnosis. Skeletal metastases developing prior to or at the same time as pulmonary metastases can be detected by bone scintigraphy. Soft tissue and pulmonary metastatic disease of Ewing's sarcoma is not detected on MDP bone scintigraphy. MDP uptake can be affected by nonspecific factors other than tumor activity. There may be marked decrease in uptake 3-4 months after treatment with radiation therapy. Intense focal uptake at tumor site within 3-4 months after treatment may be due to tumor recurrence, or complications such as infection or pathologic fracture.

MDP bone scan of primary lesion site provides

little information to predict long-term survival or disease progression in patients with non-metastatic Ewing's sarcoma. Scintigraphy with thallium-201 will more reliably show histologic tumor response. Ewing's sarcoma exhibits similar findings to osteogenic sarcoma on thallium-201 scintigraphy. Pretreatment uptake of thallium-201 is found in all extremity tumors. Because of splanchnic uptake, pelvic tumors may have equivocal uptake. Because there is not often surgical resection of the primary site, thallium may potentially provide more specificity for the presence of viable tumor compared with follow-up bone scintigraphy. Metastatic disease can be seen with thallium-201 but sensitivity data is not available. Indications for FDG-PET in Ewing's sarcoma are for detection of osseous metastases of Ewing's sarcoma, therapy monitoring and the diagnosis of recurrences <sup>(11, 12)</sup>.

While some osseous pulmonary metastases can be visualized on MDP bone scans, no other single photon scintigraphic agents are useful for this diagnosis. FDG-PET in one series of 71 combined adult and pediatric patients with osteosarcoma or Ewing's sarcoma identified FDG-PET had a sensitivity of 0.50, a specificity of 0.98, and an accuracy of 0.87 on a patient based analysis with comparable spiral CT values of 0.75, 1.00, and 0.94 respectively. Their conclusion was that at present a negative FDG-PET cannot be used to exclude lung metastases. But, because the specificity of FDG-PET is high, a positive FDG-PET result can be used to confirm abnormalities seen on thoracic CT scans as metastatic <sup>(13)</sup>.

The staging and non-invasive response assessment in rhabdomyosarcoma is challenging due to this tumor's multifocal behavior. The tumors will often accumulate Tc-99m MDP due to hypervascularity. Local bony involvement can be distinguished with 95% accuracy. Bone scans alone however do not detect soft tissue involvement by primary and metastatic disease in all cases. Thallium-201 tumor scintigraphy can be helpful in soft tissue tumors for assessment of primary and metastatic disease and response to therapy. Mild to marked thallium-201 uptake in rhabdomyosarcoma has been described in <sup>(14, 15)</sup>. FDG-PET can help in distinguishing benign soft tissue masses from

malignant lesions of soft-tissue sarcoma. FDG-PET has also been reported to be helpful in detection of unsuspected metastatic sites in patients with what is believed to be isolated disease <sup>(16)</sup>.

153- Samarium EDTMP has been reported to provide bone-specific therapeutic irradiation when used for palliation of painful bone metastases in patients with osteoblastic bone metastases from osteosarcoma <sup>(17)</sup>. Hematologic toxicity requires peripheral blood stem cell grafts to overcome myeloablative effects of the skeletal irradiation. Nonhematologic side effects are minimal. In addition this radiopharmaceutical can be imaged using a conventional gamma camera <sup>(18)</sup>.

### Lymphoma

In Lymphoma, Ga-67 imaging is well established in the staging and monitoring of response to therapy of patients with Hodgkin Disease and non-Hodgkin lymphoma (NHL). Failure to convert to a negative scan post-treatment signals a poor prognosis. In one study of 139 adult and pediatric patients with aggressive NHL, positive Ga-67 after the first cycle of treatment predicted 64% of patients who had failure of treatment. A positive study at mid-treatment predicted 77% of patients who had treatment failure <sup>(19)</sup>.

PET-FDG can be used to accurately stage, assess therapeutic response and assess for residual or recurrent disease in adults with lymphoma. Uptake in lymphoma corresponds to grade of tumor and prognosis. FDG has been shown to be more sensitive and accurate than Ga-67 in detecting splenic involvement at time of staging <sup>(20)</sup>. Tumors which are aggressive and resistant to treatment tend to show high uptake of FDG and a lower survival rate. Recent publications are identifying a similar utility of FDG-PET and PET/CT imaging in children with lymphoma <sup>(21)</sup>. Using FDG-PET as compared to CT, resulted in a higher staging in 4 of 25 patients and in a lower staging in 2 of 25 patients in a retrospective study of FDG-PET in 25 children with lymphoma <sup>(22)</sup>. As with gallium, persistent abnormal FDG-PET uptake after chemotherapy in NHL is highly predictive for residual or recurrent disease. In relapsing patients, progression free survival was significantly shorter after a positive scan than after a negative

scan <sup>(21-26)</sup>. Awareness of normal uptake patterns, variants and artifacts on PET- FDG imaging in children is important in accurate disease assessment <sup>(25)</sup>. Standardization of protocols for pediatric patients is not yet defined. The use of premedication to prevent brown fat uptake is also being evaluated as more centers perform these studies on pediatric patients <sup>(27)</sup>.

In lymphoma, residual anatomic masses are often present that cause uncertainty for the need for salvage or alternate therapy. CT and MR have limited ability to distinguish between active residual or recurrent disease or fibrosis or scar. Gallium scintigraphy is reported to be superior to CT and MR for evaluating response to therapy. Combining Ga-SPECT with MR has been reported to improve diagnostic accuracy for disease detection. PET-FDG studies can be useful to assess residual masses after chemotherapy <sup>(28)</sup>.

One of the differential diagnoses of residual anatomic mass after therapy is thymic rebound,. This physiologic thymic regeneration following chemotherapy is more common in children than in adults. Increased thymic gallium localization due to thymic regeneration and not tumor involvement has been well described with a characteristic bilobed appearance<sup>(29)</sup>. FDG-PET accumulation can also occur in normal thymus.<sup>(30-32)</sup>. The natural history of thymic rebound uptake seen scintigraphically is that it regresses after a few months and usually with 6-12 months.

### Neuroblastoma

MDP bone scintigraphy in children with neuroblastoma may show uptake in the primary tumor in 35-100% with an average occurrence of 70% <sup>(33-39)</sup>. The intensity of uptake does not correlate with grade of malignancy, prognosis, or the presence and amount of calcification present in the tumor. Metastatic uptake includes focal areas of increased radiopharmaceutical accumulation, photopenic or "cold" lesions, and symmetrical metaphyseal increased uptake. Metastatic disease detected on bone scintigraphy is often abnormal before radiographic changes are apparent. Later in the disease, however, lesions may be seen radiographically which may not be abnormal on bone scintigraphy. This reduction in uptake of

the bone-seeking radiopharmaceutical causing a false negative scan may be an altered biodistribution affect from chemotherapy.

Accurate staging is important for therapy considerations. Stage IV disease can be distinguished scintigraphically from stage IVs disease by performing MIBG scintigraphy to assess extent of disease including primary tumor, and bone disease. The better detection of metastases can upstage the disease. Tc-99m MDP bone scintigraphy is required if MIBG scan is negative or unavailable and radiographs of positive scintigraphic lesions are recommended. Sensitivity and specificity for MIBG scintigraphy are 94% and greater than 95%, respectively. Abnormal activity can be seen in the primary tumor site, and in bone, bone marrow and soft tissue metastases. In post chemotherapy evaluation of advanced neuroblastoma, MIBG scintigraphy can show tumor response appearing as a decrease in MIBG uptake and can also detect new lesions. MIBG often will detect more bone and bone marrow disease at diagnosis and in follow-up not demonstrated by bone scintigraphy, radiographs or marrow biopsy. A negative MIBG scan after induction chemotherapy is reported to be a good predictor of better disease free survival.

Uncommonly, neuroblastoma may be non-avid on MIBG scintigraphy or less avid than disease detected on bone scintigraphy. More mature forms of neural crest histology such as ganglioneuroblastoma and ganglioneuroma may have some but variable uptake of MIBG. False negative MIBG bone uptake compared to positive MDP bone uptake has been reported possibly because MDP bone scintigraphy will better reflect cortical bone disease as compared with marrow disease, suggesting a complementary role for MIBG and MDP scintigraphy <sup>(40)</sup>.

Octreotide somatostatin receptor imaging is sometimes used as an alternative to MIBG scintigraphy in children with neuroblastoma, but with a lower sensitivity of 77%. MIBG negative tumors, which are positive on somatostatin scintigraphy, are described. FDG-PET has been utilized in children with neuroblastoma. FDG accumulated in primary neuroblastoma lesions and in metastatic disease and in disease that was MIBG non-avid <sup>(12, 41-43)</sup>. Other PET

radiopharmaceuticals are also being evaluated in neuroblastoma <sup>(44)</sup>.

### **Radionuclide Therapy In Neuroblastoma**

MIBG labeled to I-131 has a relatively long half-life which varies from 2.8-8.0 days and this allows therapeutic doses of irradiation to be delivered with acceptable hemotoxicity <sup>(45)</sup>. The therapeutic use of I-131 MIBG in the treatment of neuroblastoma is well tolerated in the pediatric patient. With respect to hematologic toxicity, thrombocytopenia is the main side effect and is dependent on the status of the bone marrow at the time of treatment. Patients who have extensive bone marrow invasion and have been treated with chemotherapy at the time of treatment are much more likely to develop toxicity, and bone marrow suppression may be permanent. Patients who receive MIBG therapy as front line therapy are much less likely to suffer this complication since they do not yet have substantial marrow involvement <sup>(46-49)</sup>. Post therapy whole body I-131 MIBG scans may demonstrate sites of marrow involvement not appreciated on pre-treatment scans.

Initially treatment of neuroblastoma with I-131 was limited to patients with advanced disease who had failed to achieve any significant success with other more established treatment modalities. Most series consisted almost exclusively if not exclusively of patients with progressive stage IV disease. Despite that, early cumulative results in 276 patients demonstrated a positive response in 35% <sup>(50)</sup>. MIBG had proven itself to have potential in palliation and improvement in the quality of life in these seriously ill children. I-131 MIBG therapy has also been used as a front line therapy in patients with advanced neuroblastoma at the time of diagnosis. In a series of 49 patients with stage 3 and 4 neuroblastoma one year survival was 65% and 5 year survival was 38% <sup>(51)</sup>. Toxicity was not severe with thrombocytopenia being the main complication. A recent study using multiple infusion of I-131 MIBG showed increasing response but did show hematologic toxicity <sup>(52)</sup>. Studies for treatment with I-131MIBG in combination with myeloablative chemotherapy and hematopoietic stem-cell rescue has also proven feasible with acceptable toxicity <sup>(52, 53)</sup>.

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# The Value of Molecular Diagnostic Testing in Paediatric Onco-pathology

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The pathologic diagnostic process has evolved over time to incorporate data generated from novel assays. Initial categorization of lesions was based on combined gross appearance and histological patterns, as reflected in haematoxylin and eosin-stained tissue sections on glass slides. Fine structural analysis by electron microscopy became common-place in the 1980's and was then supplemented by immunohistochemical characterization, particularly of tumours. Immunohistochemical evaluation is a key component of tumour interpretation, with ever-increasing panels of stains to assist in characterising a lesion. Recognition of recurrent genetic aberrations in tumours particularly, has led to routine genetic analysis of such cases through cytogenetic and/or molecular testing. Apart from providing information useful for tumour diagnosis, this also may provide prognostic and, in an era of targeted therapeutics, even predictive data. Thus the pathologist now finds him/herself in a position where they can render not only a 'straight diagnosis', but also provide further data potentially of considerable importance for the patient's management.

In this lecture, I will discuss the types of genetic abnormalities that may be of diagnostic value, the means by which some of these abnormalities might have their effects, what modalities (with emphasis on molecular means) are currently in use to test for these abnormalities and what the value of such assays might be in terms of diagnosis/classification, prognosis, and therapeutic prediction/monitoring.

The types of genetic abnormalities that may be of diagnostic relevance include chromosomal translocations, alterations of gene dosage and specific gene mutations.

Cytogenetic evaluation, particularly of

haematopoietic and mesenchymal tumours, has led to the recognition of recurrent aberrations in the form of chromosomal translocation. Particular translocations tend to segregate with specific tumour types and so a diagnostically useful list may be generated. Identification of chromosomal translocations has led to re-classification of certain tumours.

A chromosomal translocation may confer oncogenic properties through a variety of means. One well-established means is through the production of a 'chimeric' or 'fusion' oncogene which acts as a novel transcription factor. A further possibility is through constitutive signal activation, while another mechanism is through ectopic expression of a growth factor.

An example whereby translocation produces a chimeric gene acting as a transcription factor is the t(11;22)(q24;q12) occurring in Ewing sarcoma/peripheral primitive neuroectodermal tumour (pPNET). Fusion of the DNA-binding domain of Fli-1 with the transcriptional activating domain of EWS confers oncogenic capacity. A similar mechanism of oncogenesis occurs in DSRCT, clear cell sarcoma, myxoid liposarcoma, low-grade fibromyxoid sarcoma, and angiomatous fibrohistiocytoma, all of these having in common the fact that one partner in the fusion gene, EWS or TLS belongs to the TET gene family.

The latter translocation, t(12;15)(p13;q25) produces a fusion gene ETV6-NTRK3 resulting in constitutive tyrosine kinase activation. Similar tyrosine kinase activation occurs in TPM3/4-ALK and TPM3-NTRK1 fusions. The same chromosomal translocation can be demonstrated in distinct tumours even tumours of different lineage, as best exemplified by t(12;15)(p13;q25), which has now been identified in tumours of mesenchymal, haematopoietic and epithelial lineages; the TPM-associated

translocations between them also spanning the spectrum of mesenchymal, haematopoietic and epithelial malignancies.

Chromosomal translocation may produce ectopic growth factor activation, as in t(17;22)(q21;q13.1) fusing Col1a1 with PDGFRb.

Apart from the diagnostic value of identifying chromosomal translocations in human tumours, there may also be prognostically important information provided, occasionally in the fine detail of the translocation, identifiable only through molecular means. Probably the best established translocation-associated prognostic factor in paediatric solid malignancy is the t(1;13) versus t(2;13) status of alveolar rhabdomyosarcoma. There have been reports that the so-called type I transcript of EWS-Fli1 is associated with better prognosis than the other transcript types. Similarly, SYT-SSX1 has variably been reported as being associated with better and worse outcomes than the SYT-SSX2 transcript in synovial sarcoma. Beyond this, attempts have also been made to link transcript type with histological subtype in certain tumours.

Gene dosage alterations may occur through larger deletions or amplifications involving whole chromosomal regions, or involve alterations in single gene copy number and/or expression levels. Deletion of 22q is considered diagnostic of the atypical teratoid rhabdoid tumour (ATRT) in the differential of paediatric embryonal CNS tumours. The latter abnormalities may be diagnostically useful, but particularly tend to be of prognostic value. N-Myc status is well-established as a prognostically important parameter, while gain of 17q and deletion of 1p are similarly of prognostic importance in neuroblastoma. There is evidence that Myc status is important in medulloblastomas also. Evidence points to gains and losses at specific loci also being of prognostic value in Wilms tumour, medulloblastoma and osteosarcoma.

Specific gene mutations generally tend not to constitute diagnostically useful information, but may be prognostically and increasingly of therapeutic significance.

There are various modalities by which these miscellaneous diagnostic- and prognostically important genetic characteristics might be

assayed. For chromosomal translocations, the best global overview of the genetic make-up of a tumour comes from karyotypic analysis, typically through G-banding. The chromosomes are all reviewed without bias and so, unanticipated aberrations should also be detected. The advantage is that one has a complete set of data, albeit on a gross level. Molecular testing on the other hand, through PCR-based assays, addresses a specific and narrow question, implying identification, at most of just the abnormality sought, while potentially missing others. The information generated is however potentially more detailed, insofar as sequence information indicating the precise breakpoint for example becomes available. In practical terms, cytogenetic analysis is slow, expensive and has a considerable propensity to failure, given the difficulty in culturing primary tumour cells. PCR-based assays on the other hand, tend to provide rapid results and while preferably performed on fresh template, can potentially also work out of paraffin-embedded material, meaning that one can return to cases where genetic work-up was initially omitted, for whatever reason.

Alterations in gene dosage, notably N-myc amplification studies have traditionally relied on Southern analysis, however this does not take into account intratumoural heterogeneity – fluorescence in-situ hybridization (FISH) is therefore more useful to establish the status in individual cells, but determination of copy number remains difficult by this means. Probably the best combination testing for amplification is quantitative PCR in conjunction with FISH. For gross deletions or amplifications, either cytogenetic evaluation or FISH analysis may be applied. This is a ready means of diagnosing del 22q in rhabdoid tumours and ATRT for example. Determination of gene expression level is best through RT-Q-PCR.

The search for specific gene mutations is not so common-place in paediatric solid tumour evaluation. However, there are some tumours where specific gene mutations are worth seeking in order to establish the likelihood of benefit from targeted therapy. The paradigm of this is of course the gastrointestinal stromal tumour with c-kit mutation where therapy with imatinib mesylate (STI-571) is indicated. Detailed

information regarding the specific mutation in the c-Kit gene generally requires a PCR-based assay with sequencing of the amplified product. p53 mutation detection is also considered of value.

The purpose of genetically evaluating tumours ultimately is to facilitate development of targeted therapy. 'Molecular therapy' may target any of the above-mentioned cancer cell-associated aberrations, including fusion products of chromosomal translocations or may be directed to inhibit constitutively activated kinases by a variety of different strategies.

In summary then, molecular assays may be of value in diagnostic terms, in classification of tumours, in prognosis and in therapeutic prediction.

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# Supportive care - Present use of biologicals in supportive care

Cesaro Simone

**K**ey words: growth factors, G-CSF, filgrastim, pegfilgrastim, erythropoietin, darbepoetin,

Dose and schedule are important factors in the therapy of cancer. Animal model and clinical data show that there is a significant correlation between dose-intensity and survival; moreover, the shortening of interval between chemotherapy cycles prevents from tumor regrowth and maximize the effect of chemotherapy on cancer cells (Gregory SA, Trumper L, 2005). Most of anti-cancer drugs are non-selective and act indiscriminately on dividing cells. Hematological toxicity is one of the most important limiting factor for the protocols that are based on the dose-escalation or the dose-density of chemotherapy. This result in peripheral blood cytopenia (neutropenia, anemia and thrombocytopenia), higher transfusion requirement, severe infectious complications, prolonged hospitalization and higher health care expenses. The use of hematopoietic growth factors such as the granulocyte colony-stimulating factors (G-CSF) filgrastim or lenograstim and erythropoietin represented a step forward to reduce the hematological toxicity of high-dose chemotherapy and to manage the frequent infectious complications.

## **G-CSF**

G-CSF is a natural cytokine that acts on committed myeloid progenitor cells. Its secretion is stimulated by infections or by the reduction of mature myeloid cells, as a result of bacterial lipopolysaccharides and chemotherapy, respectively (Lieschke GJ, Burgess AW, 1992); so the serum G-CSF concentration increase from approximately 25 pg/ml of healthy people to 1,000 pg/ml of patients with severe infections or after stem-cell transplantation (Kawakami et al, 1990).

Several studies showed a clear benefit of the use of G-CSF in terms of reduction of length of severe neutropenia, incidence of infections, use of intravenous antibiotic and duration of hospitalizations. Overall, G-CSF facilitates the delivery on time of chemotherapy planned dose but the high costs of acquisition raised the issue of its appropriate use. The guidelines of The American Society of Clinical Oncology (ASCO), updated in 2000, indicated the settings where the use G-CSF is recommended on the basis of the available clinical data. In particular, G-CSF is recommended as primary prophylaxis of febrile neutropenia in patients with an expected incidence of chemotherapy-induced neutropenia greater than or equal to 40%; in the treatment febrile neutropenia in patients at high risk of severe infections (sepsis, pneumonia, fungal infections); after high-dose chemotherapy with autologous progenitor stem-cell rescue; in the mobilization of peripheral blood progenitor cells (PBPCs); in patients with acute myeloid leukemia to reduce the neutropenia of the post-induction chemotherapy; and in patients with acute lymphoblastic leukemia to reduce the neutropenia that follows the induction chemotherapy. The adult indications of the use of G-CSF are generally extended to paediatric patients, though less data are available (Ozer et al, 2000).

Recently, new interest in G-CSF therapy has been obtained with the introduction of pegfilgrastim, the pegylated form of filgrastim (Waladkhani AR, 2004). Filgrastim, the recombinant human G-CSF, is a relatively small protein that is rapidly eliminated from the body via the kidneys. The short half-life, about 3.5 hours, requires its daily administration by intravenous or subcutaneous injection until the recovery to normal values of the absolute neutrophil count. Pegfilgrastim consists of a 20-kDa polyethylene glycol molecule covalently

bound to the N-terminal amino group of filgrastim molecule. Polyethylene glycol molecule are pH-neutral, non-toxic, water soluble polymers that confers to pegfilgrastim a larger volume and a slower renal clearance; as a result the half-life of pegfilgrastim is 35 hours. The most important route of elimination of pegfilgrastim is the so-called neutrophil-mediated clearance: after binding with the G-CSF receptor on surface of neutrophils, the molecule is removed from circulation and the resulting molecule-receptor complex is internalized and metabolized. The neutrophil-mediated clearance is a process slower than renal clearance and in healthy volunteers this molecule produced a sustained neutrophil count for 9-10 days (Molineux et al, 1999). The study performed on neutropenic cancer patients showed that pegfilgrastim reached a peak approximately 24 hours after injection, remained high for all the neutropenic period without daily fluctuation and declined as the patient recovered the baseline count of neutrophils. This favourable kinetic provides a patient's tailored protection from severe neutropenia and a smooth recovery of neutrophil levels.

Several phase II-III studies with pegfilgrastim has been performed in lung cancer, breast cancer, and lymphoma (Waladkhani AR, 2004; Biganzoli et al, 2004; Holmes et al, 2002; Green et al, 2003; Siena et al, 2003) and the results are summarized as follows: a) the fixed dose of 6 mg (or 100 mg/kg) of pegfilgrastim, administered once per chemotherapy cycle, is equivalent to the 5 mg/kg-daily-dose of filgrastim, administered for 10-11 days, with respect to the incidence and duration of severe neutropenia, and the median time to absolute neutrophil count recovery; b) no dose-limiting toxicities were observed with pegfilgrastim, and the safety profile or the incidence of adverse events was similar to that of filgrastim, including bone pain; c) no effect of body weight was found on duration of severe neutropenia; d) a lower risk of febrile neutropenia was observed in patients who received pegfilgrastim than those given daily filgrastim: 11% vs 19% (relative risk 0.56, C.I. 0.35-0.89,  $p < 0.005$ ); f) a trend towards a lower risk of hospitalization and use intravenous antibiotics was observed in patients treated with pegfilgrastim. A recent randomized study in

breast cancer patients who underwent moderately myelosuppressive chemotherapy regimen showed that the use of pegfilgrastim compared to placebo was associated to a lower incidence of febrile neutropenia (1% vs 17%), febrile-neutropenia-related hospitalization (1% vs 14%) and intravenous antibiotic use (2% vs 10%) These findings demonstrated that the use of pegfilgrastim reduced significantly the incidence of infectious complications also in the patients with a moderate risk of febrile neutropenia (10-20%) and raised the issue of its use as primary prophylaxis out of the current guidelines of ASCO (Vogel et al, 2005). Phase II studies recently reported that pegfilgrastim was effective both in mobilizing a sufficient number of CD34+ peripheral stem cell in patients with myeloma and lymphoma (Isidori et al, 2005; Steidl et al, 2004) and in decreasing the duration severe neutropenia and febrile neutropenia after autologous peripheral blood stem cell transplantation (Staber et al, 2005). These data deserve a further validation by prospective randomized study. In conclusion, pegfilgrastim offers a simplified dosing regimen that is more convenient for nurses and patients but its potentiality warrants further investigation especially in setting where no data are still not available or conclusive (children, stem cell mobilization, autologous stem cell transplantation).

### **Erythropoietin (EPO)**

Anemia is a common complication in patients treated with chemotherapy for cancer. Its occurrence may delay the chemotherapy schedule, affect negatively the quality of life (QoL) and compromise the anti-tumour activity of radiotherapy and chemotherapy (Ludwig H, Fritz E, 1998; Crawford et al, 2002). Prior to 1980s, the treatment of cancer-related anemia was based only on red blood cell (RBC) transfusion when the hemoglobin levels fell below 8-9 g/dl. The introduction of recombinant human erythropoietin in the '80s gave the opportunity to reduce the need for RBC transfusions and to improve overall QoL and possibly prognosis of patients. EPO is a protein synthesised in the kidney and, to a lesser extent, in the liver that binds erythropoietin receptors on surface of bone marrow red cell precursors (BFU-e, CFU-e, erythroblasts) and promotes

erythropoiesis. This glycoprotein hormone has a molecular weight of 34 kDa and consists of 165 aminoacids; carbohydrates represent around the 40% of the molecule. Three recombinant human EPO has been approved for anemia in cancer: epoetin alfa, epoetin beta and darbepoetin alfa (Engert A, 2005). Evidence-based guidelines have been published by ASCO and EORTC about the use of the recombinant human erythropoietin in patients with cancer. The major goals of the erythropoietin therapy is the correction of chemotherapy-related anemia (defined as Hb level < 9-11g/dl), prevent transfusions and possibly improve the QoL. The recommended dose of epoetin alfa and beta is 150 IU/kg three times a week for a minimum of 4 weeks that can be escalated to 300 IU/kg three times a week for other 4-8 weeks in those patients who do not respond to the initial regimen. An alternative schedule is the administration of 30-40.000 IU once a week in order to improve patient compliance (Rizzo et al, 2002; Bokemeyer et al, 2004). Darbepoetin alfa is a biochemically distinct erythropoietin characterized by an increased carbohydrate content, a major number of sialic acid molecules and a higher molecular weight; these properties determine a longer half-life (about 49 hours) and an increased biological activity compared to epoetin alfa or beta. The recommended dose of darbepoetin is 2.25 ug/kg per week but a dose finding study in patients with solid tumours showed that the most effective weekly dose is 4.5ug/kg (Glaspy et al, 2002). In the same study, the administration of 9 ug/kg every 2 weeks had a comparable efficacy to the weekly dose of 4.5ug/kg. Other authors found that the doses of 12 ug/kg and 15 ug/kg of darbepoetin alfa allow to maintain the efficacy of darbepoetin despite a longer interval of administration, 3 and 4 weeks, respectively (Kotasek et al, 2002; Glaspy et al, 2003). A recent meta-analysis including 27 prospective randomized trials published between 1985 and 2002 demonstrated that the use of recombinant human erythropoietin reduced significantly the risk of RBC transfusion, mainly in patients with solid tumours and gives some evidence for improving QoL and survival (Bohlius et al, 2005). Despite these favourable data, recombinant human erythropoietin is not routinely used in cancer patients for several reasons: limited data (children), best dosing

schedule not defined yet, slow onset of response, costs, no clear impact on survival and risk of thrombovascular events when used to correct Hb levels beyond anemia.

In conclusion, the development of long-acting darbepoetin gives the opportunity to simplify the management of chemotherapy-related anemia but more data are needed to assess the real cost/benefit ratio and impact on outcome. -

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## Future – biologicals in development

Lars Wagner

**A**lthough steady improvements have been made in cure rates for many types of paediatric cancers, the toxicity of treatment can be substantial. Knowledge about underlying mechanisms of toxicity continues to grow, potentially allowing for specific interventions that may be more effective at ameliorating side effects of therapy. This review will summarize the development of selected new agents which target three key chemotherapy toxicities: diarrhea, nausea, and neutropenia. In most situations, there is limited information for these agents in children compared to adults; however, it is important for paediatric oncologists to be aware of new developments in this field so that promising agents can be rapidly moved into paediatric clinical trials.

### Diarrhea

Interest in controlling chemotherapy-related diarrhea has increased as the camptothecin drug irinotecan (CPT-11) is being utilized more for paediatric tumors. This drug has now been approved for treatment of colon cancer, and has also demonstrated activity against rhabdomyosarcoma, neuroblastoma, and high-grade astrocytoma. The relatively mild myelosuppression seen with irinotecan makes it attractive for use in combination with other agents, and given the wide range of activity, its use is expected to increase in the future. However, the diarrhea caused by irinotecan is considerable and can significantly affect patient care by resulting in treatment delays, dose reductions, or discontinuation of therapy. Because diarrhea is often dose-limiting, and because reducing diarrhea may improve irinotecan dose intensity and potentially result in better antitumor activity, there has been substantial efforts to identify effective interventions for this problem.

There are two patterns of diarrhea seen most

commonly after treatment with irinotecan. Early-onset diarrhea usually occurs within 4 hours of administration, and is usually accompanied by flushing and cramping. This type of diarrhea is associated with higher doses of irinotecan, and can be readily treated and even prevented with the use of anticholinergic drugs such as atropine. The more common late-onset diarrhea from irinotecan occurs during or after the second week of treatment, and is often more refractory to treatment. Additional complications can develop in these patients, including debilitating crampy pain, dehydration and hypotension, intestinal bleeding or perforation, and sepsis, particularly in neutropenic patients. The likelihood of developing diarrhea may be in part related to the dosing schedule of irinotecan; for example, patients receiving protracted dosing of irinotecan, such as the dx5x2 schedule used in many paediatric protocols, are more likely to be limited by diarrhea instead of neutropenia<sup>(1)</sup>.

Agents that appear to be helpful in reducing irinotecan-associated diarrhea are discussed below. Some involve general symptomatic treatment, while others are specifically targeted for irinotecan. When evaluating these different strategies, it is important to consider not only efficacy but also the feasibility of administering these medicines to children, who may be receiving more protracted dosing of irinotecan. Table 1 briefly summarizes pertinent information regarding each of these approaches.

### Non-Specific Measures for Treating Chemotherapy-Induced Diarrhea

The most commonly used medicine for treating irinotecan-induced diarrhea is **loperamide (Imodium®)**, an opioid antimotility agent which decreases intestinal peristalsis. This drug has been recommended by the American Society of Clinical Oncology (ASCO) expert panel as the first agent to use with chemotherapy-induced

**Table 1. Summary of agents used to control irinotecan-induced diarrhea**

Agent	Mechanism	Administration	Feasibility	Comments	References
Loperamide	Antimotility agent	Oral, up to q2hr after each loose stool	Requires frequent administration	Most common and recommended agent	2-4
Diphenoxylate	Antimotility agent	Oral tid	Feasible	May be less active than loperamide	5
Acetorphan	Enkephalinase inhibitor	Oral tid	Feasible	Not available in US	6, 7
Octreotide agent	Anti-secretory in children	Subcutaneously tid second-line agent	Less feasible	Recommended	2, 8
Octreotide LAR	Anti-secretory agent	Intramuscularly q 28 days	More feasible than standard dosing	No paediatric data	9
Budesonide	Anti-inflammatory	Oral tid	Well tolerated	Unknown safety with protracted administration	10
Thalidomide	Immunomodulator	Oral qHS	Well tolerated	Has independent antitumor activity	11
Activated charcoal	Impairs absorption	Oral tid	Unlikely to be tolerable in children	Difficult with protracted dosing	14
Alkalinization	Converts SN-38 to less toxic form	Oral tid	Unknown tolerability in children	Difficult with protracted irinotecan	12, 13
Kampo	Glucuronidase inhibition	Oral tid	Unknown tolerability in children	Drug availability issues	15
Neomycin	Kills glucuronidase-producing bacteria	Oral tid	May be unpalatable in children	Difficult with protracted irinotecan	16, 17
Cefixime	Kills glucuronidase-producing bacteria	Oral qd	Very feasible, well tolerated	Only regimen studied with protracted irinotecan	18, 21

diarrhea<sup>(2)</sup>. Aggressive dosing at the start of first loose stool appears to be most effective and clearly reduces the incidence of severe diarrhea, but even with intensive loperamide dosing (2 mg q 2 hrs), up to 30% of patients in Phase II trials of irinotecan in the US develop severe diarrhea<sup>(3, 4)</sup>. So use of loperamide is important but not always sufficient in protecting patients against serious toxicity. Prompt initiation of loperamide after the first loose stool may also be important, and proper counseling and preparation may optimize use of this drug. **Diphenoxylate** (Lomotil®) has a similar mechanism of action, but crossover studies suggest it may not be quite as effective as loperamide<sup>(5)</sup>.

**Acetorphan (Tiorfan®)** is an oral enkephalinase inhibitor which can be safely combined with loperamide, and this two-drug approach was more effective than either agent alone in a randomized trial of adults receiving irinotecan<sup>(6)</sup>. However, single-agent prophylactic use did not reduce diarrhea in one study<sup>(7)</sup>. Drug availability issues have limited wide-spread international use of this agent.

**Octreotide (Sandostatin®)** is an octapeptide which acts directly on epithelial cells to reduce secretion of various pancreatic and gastrointestinal hormones. This drug prolongs intestinal transit time, improves absorption and decreases secretion of electrolytes, and has been approved in the US for treatment of diarrhea from malignant carcinoid tumors. Octreotide 150 micrograms is usually given subcutaneously three times daily, with doses of 500 micrograms or higher perhaps being more effective in adult studies<sup>(8)</sup>. This drug is recommended by the ASCO expert panel as a second-line agent for patients who are poorly controlled with loperamide<sup>(2)</sup>. A long-acting intramuscular preparation given once monthly is also commercially available, and this more conveniently administered product has had some effectiveness in a small study<sup>(9)</sup>.

Agents which reduce inflammatory changes in the intestine may also be effective in reducing diarrhea. Small trials of oral administration of the steroid **budesonide**<sup>(10)</sup>, or the immunomodulating agent **thalidomide**<sup>(11)</sup>, have

shown some success in reducing irinotecan-associated diarrhea. The use of thalidomide is particularly interesting, since this agent has been associated also with antiangiogenic and antitumor effects; accordingly, a Phase II trial of this combination is planned.

### Irinotecan-Specific Approaches to Reduce Diarrhea

Chemotherapy-induced diarrhea is a complicated, multifactorial process resulting in an imbalance between absorption and secretion. In the case of irinotecan, this imbalance is triggered by an accumulation of the active metabolite SN-38 in the intestine, which is the result of the following steps:

1. The prodrug irinotecan is converted by endogenous carboxylesterases to the active metabolite SN-38.
2. SN-38 is normally detoxified by the liver through the process of glucuronidation, and the inactive compound SN-38-glucuronide (SN-38-G) is then secreted into the intestine and eliminated.
3. However, enteric bacteria can produce glucuronidases which remove the glucuronide moiety and convert the compound back to active SN-38 in the gut, which then causes direct cytotoxicity and results in secretory diarrhea.

Understanding of this process provides insight into opportunities for more specific management of irinotecan-related diarrhea.

Irinotecan and its metabolites SN-38 and SN-38-G all have lactone rings which are in equilibrium after pH-dependent hydrolysis. The closed lactone formation is more prevalent at an acid pH, while the open carboxylate formed is preferentially formed at neutral or basic pH. Because this carboxylate form has less topoisomerase I inhibition and therefore is less toxic, selective conversion down this pathway may be important in reducing diarrhea caused by the SN-38 formed by deglucuronidation. Also, acid pH results in more passive absorption of intestinal SN-38, and so a basic environment in the intestine may reduce additional accumulation of this metabolite. In a crossover study of 10 colon cancer patients using this alkalinization approach, oral **sodium bicarbonate** reduced

incidence of diarrhea; importantly, there was no interference observed with irinotecan pharmacokinetics<sup>(12)</sup>. An additional report using a multivariate analysis has shown reduction in risk of > grade 2 diarrhea in adults receiving oral alkalinization along with magnesium oxide to produce more regular bowel movements and avoid constipation that would prolong exposure of SN-38 to gut tissue<sup>(13)</sup>.

Agents which reduce adsorption of SN-38 in the intestine may also be effective. **Activated charcoal**, widely used as an emergency antidote for toxic ingestions, markedly reduced irinotecan-associated diarrhea in a recent crossover study<sup>(14)</sup>. Charcoal was diluted in water for a total volume of 30 ml, given before and for 6 doses after irinotecan, with good tolerability in this adult study.

Because glucuronidases contribute to irinotecan-induced diarrhea, one approach has been to directly inhibit this enzyme using **Kampo medicine Hangeshashin-to (TJ-14)**. This compound contains baicalin, and appears to be effective in preclinical studies as well as a small Japanese clinical trial of adults receiving cisplatin and irinotecan<sup>(15)</sup>. Another approach is to use antibiotics to selectively eradicate the bacteria which produce this enzyme. The use of oral **neomycin**, a non-absorbable broad-spectrum antibiotic, is effective for this purpose in both mouse models<sup>(16)</sup> and clinical trials of adults receiving irinotecan<sup>(17)</sup>. However, thrice daily administration of this unpalatable drug is not likely to be feasible in children who are receiving protracted irinotecan. As an alternative, investigators at St. Jude Children's Research Hospital have pioneered the use of **cefixime**, a commercially available oral cephalosporin with activity against Gram negative aerobic bacteria implicated in the production of glucuronidases. Cefixime is well tolerated and can be given once daily, starting up to 5 days before chemotherapy administration and continuing daily throughout the course of protracted irinotecan. Using cefixime combined with prn use of loperamide, Furman et al. have been able to increase the maximum tolerated dose (MTD) of oral irinotecan from 40 mg/m<sup>2</sup>/day without antibiotics to 60 mg/m<sup>2</sup>/day with antibiotics, using the dx5x2 schedule of irinotecan given orally<sup>(18)</sup>. This finding is important because systemic SN-38 exposure from oral irinotecan at this higher dose is now

comparable to that seen with intravenous irinotecan, potentially allowing for much greater patient convenience while still achieving reasonable drug exposures. Because the mechanism of diarrhea is similar whether the irinotecan is given orally or intravenously, an additional study is ongoing which combines cefixime with intravenous irinotecan; preliminary results suggest that doses of at least 150% of the usual MTD are tolerable when using cefixime, and further escalation continues (C. Rodriguez-Galindo, personal communication). Finally, in the recently completed Children's Oncology Group (COG) trial for metastatic rhabdomyosarcoma, cefixime was successfully used as secondary prophylaxis to help patients who previously experienced severe diarrhea tolerate additional standard doses of irinotecan.

In summary, many different approaches have been effective in ameliorating diarrhea from irinotecan. Few clinical comparison studies have been done<sup>(6)</sup>, although in preclinical models the use of cephalosporins was more effective than charcoal<sup>(19)</sup>. Cefixime, and the related cephalosporin cefpodoxime, are the only agents to have been evaluated in the setting of protracted irinotecan, which is the dosing schedule proven superior for treatment of paediatric tumors in preclinical studies<sup>(20)</sup>. Although there is the potential problem that cefixime may lead to the development of resistant infection, this problem has not been seen in 3 ongoing and 2 completed small paediatric studies<sup>(18,21)</sup>. Because of the feasibility and efficacy of this drug in combination with loperamide, the use of cefixime is being planned in three additional Phase I studies to be run through the COG.

What may ultimately be of greatest benefit is the ability to target interventions to those most likely to develop diarrhea. Genetic polymorphisms in the promoter region of the *UGT1A1* gene have been identified which correlate to some degree with toxicity<sup>(22)</sup>, and the validity of these observations is currently being tested in paediatric patients receiving protracted irinotecan.

### Chemotherapy-Induced Nausea

Nausea and vomiting are among the most common and dreaded side effects of chemotherapy, and can have a significant toll

on the medical condition and psychological well-being of cancer patients. The use of 5-hydroxytryptamine type-3 (5-HT<sub>3</sub>) receptor antagonists has been a major advance in the field because of their effectiveness and safety. However, many patients still struggle with debilitating nausea and vomiting, and the search continues for agents which are even more active in controlling this side effect.

While there are likely many mechanisms causing chemotherapy-related nausea, one primary route is chemotherapy inducing damage to the gastrointestinal mucosa, causing the release of serotonin (5-HT) from intestinal enterochromaffin cells. Serotonin then activates 5-HT<sub>3</sub> receptors on vagal afferent nerve fibers, which send signals to the vomiting center in the brainstem and stimulates emesis. There are four so-called "first generation" 5-HT<sub>3</sub> receptor antagonists currently approved in the US: **ondansetron (Zofran®)**, **granisetron (Kytril®)**, **dolasetron (Anzemet®)**, and **tropisetron (Navoban®)**. According to current evidence-based consensus guidelines, these four agents are considered therapeutically equivalent and interchangeable when used at equipotent doses. Although the usual dosing frequency of ondansetron is the shortest, this agent is available as an oral liquid or a dissolvable tablet, which may be well suited to paediatric patients.

**Palonosetron (Aloxi®)** is a "second generation" selective 5-HT<sub>3</sub> receptor antagonist, with two potential advantages over first generation agents. First, palonosetron has a 100-fold stronger binding affinity for the 5-HT<sub>3</sub> receptor. Second, this agent has a dramatically longer half-life of up to 40 hours, potentially leading to better control of delayed emesis. There have been three large adult Phase III trials comparing palonosetron to standard 5-HT<sub>3</sub> receptor antagonists<sup>(23-25)</sup>. In all studies there was numerical and sometimes statistically significant improvement in acute and/or delayed emesis for patients receiving single intravenous doses of either 0.25 or 0.75 mg of intravenous palonosetron given before moderately or highly emetogenic regimens. The toxicity of this drug is comparable to other 5-HT<sub>3</sub> receptor antagonists, with mild headache and constipation occurring in 10% or less of patients. Additional studies combining this agent with dexamethasone are

ongoing. There have been no reports to date of its use in children, or in multi-day regimens commonly used in paediatric oncology.

**Aprepitant (Emend®)** is a new substance P (protachykinin 1) inhibitor that is discussed in detail elsewhere. Adult studies show efficacy in controlling acute and delayed emesis when combined with ondansetron and dexamethasone <sup>(26, 27)</sup>. However, as with palonosetron, there is no direct information regarding how this agent works for paediatric patients receiving multi-day therapy. In addition, this drug is metabolized by CYP3A4 of the cytochrome P450 system, and so may affect clearance of dexamethasone, vinca alkaloids, and other chemotherapy agents. Further study is needed to clarify how this potentially effective agent may be best applied.

Other agents being used or developed include **olanzapine (Zyprexa®)**, an atypical antipsychotic with affinity for dopamine and other receptors. This drug has been used for controlling nausea from chronic opioids, and is now being studied in patients receiving chemotherapy <sup>(28)</sup>. Side effects include somnolence, hypotension, constipation, and dizziness, but not extrapyramidal effects. **Metopomazine**, a phenothiazine derivative widely used in Europe, also has high affinity for dopamine receptors, but is not associated with extrapyramidal effects. In fact, one of its main side effects is orthostatic hypotension due to adrenergic receptor antagonism. Metopomazine works well in combination with conventional 5-HT<sub>3</sub> receptor antagonists and/or steroids, and can be given intravenously or orally in doses divided 2-4 times per day. There have been no published paediatric trials for this agent, which is not currently available in the US.

### Chemotherapy-Induced Neutropenia

The use of the neutrophil growth factor **filgrastim (G-CSF, Neupogen®)** has become commonplace following many of the myelosuppressive regimens used in paediatric oncology. Filgrastim is commonly given as a daily subcutaneous injection starting the day after completing chemotherapy and continuing until the neutrophil count has recovered from its nadir. **Pegfilgrastim (Neulasta®)** is a pegylated formulation of filgrastim in which a polyethylene

glycol molecule is bound to the N-terminal amino group of filgrastim, dramatically prolonging its half life. This creates a larger molecule which is less readily cleared by the kidneys. Instead the clearance of pegfilgrastim is accomplished primarily by neutrophils, which bind the filgrastim moiety and internalize it. This slower clearance results in a sustained duration of action. In healthy volunteers, a single dose of pegfilgrastim causes persistently high levels of neutrophils for 9-10 days. Its half-life is extended even further in patients who are neutropenic, and high levels of drug remain until neutrophil recovery. This self-regulating "on/off switch" also results in a smooth recovery of neutrophils following the nadir, as drug is increasingly cleared by the rising neutrophil count.

Two adult Phase III studies have confirmed that a single dose of pegfilgrastim, given at 100 g/kg or at a set dose of 6 mg, is as effective as an average of 11 doses of G-CSF in ameliorating chemotherapy-induced neutropenia <sup>(reviewed in 29)</sup>. In fact, when pooling data together from these studies, the incidence of febrile neutropenia was in fact lower with pegfilgrastim than with G-CSF (11% vs. 19%,  $p < 0.05$ ). Importantly, the toxicity profile is similar, with mild-to-moderate bone pain occurring in about one-fourth of patients with each drug.

Paediatric experience with pegfilgrastim has been quite limited. An initial pilot trial has been reported in abstract form <sup>(30)</sup>, describing results similar to the adult studies. Specifically, pegfilgrastim at 100 g/day given once-per-cycle was as safe and effective as standard-dose G-CSF in 14 sarcoma patients ages 12-21 years who were receiving multiple courses of myelosuppressive therapy. Further study is planned to include more patients under 12 years of age, and it is likely that the current COG intermediate-risk rhabdomyosarcoma study will be extended to allow for randomized evaluation of pegfilgrastim in a 6:1 patient ratio following VAC chemotherapy.

In conclusion, there are a variety of new agents becoming available which have great promise to reduce various toxicities of current treatments. The challenge for paediatric oncologists lies in identifying the most active and feasible drugs and evaluating them in well-designed clinical trials that accurately assess their safety and utility.

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# Solid Tumors - role of biological agents in therapy

Kate Matthay

New tumor-targeted biological approaches to the therapy of childhood solid tumors have the promise to improve the survival of children with advanced or refractory malignancy with less acute and long term toxicity. The challenge that remains is still similar to that with chemotherapy: identifying metabolic and genetic pathways and targets that are sufficiently different or amplified in the malignant cells to allow interruption without disruption of normal cell processes, and to then synthesize molecules that will inhibit these targets without other non-specific toxicity. Great expectations were raised by the success in two forms of leukemia of agents that target a specific genetic translocation: the older results with induction of remission by all-trans retinoic acid, targeting the retinoic acid receptor, disrupted by the 15;17 translocation in acute promyelocytic leukemia, and the dramatic responses to imatinib, a small molecule targeting the bcr-abl translocation in CML. Thus far, the search for similar “druggable” genetic targets in paediatric cancers has not yet resulted in such dramatic results, though many genetic aberrations that might provide potential targets have been identified, such as the translocation with its cloned EWS-Fli1 protein in Ewing’s sarcoma or MYCN gene amplification in neuroblastoma. The far-reaching technologies brought forward by the Human Genome Project of mRNA profiling by micro-arrays now allows description of the gene expression profile of each tumor in great detail, which can then be correlated to biological or clinical characteristics of the tumors to help select new targets.

Biologicals are defined here as agents that are either uniquely or partially tumor-specific, rather than indiscriminately cytotoxic. Such agents may be directed either at the tumor itself, such as a surface receptor, or a unique genetic or metabolic feature within the cell, or at the

microenvironment of the tumor. It is now clear that what happens outside the tumor cell boundaries, in the tumor microenvironment, can have a significant impact on tumor progression. A variety of host-derived cells contribute to the tumor microenvironment including endothelial cells, pericytes and smooth muscle cells, fibroblasts and inflammatory cells like neutrophils, tumor associated macrophages, mast cells and T and B lymphocytes. Examples of how current paediatric solid tumor studies are exploiting these two approaches are discussed below. Only a few of these studies have yielded results to date, as most of these agents are still in Phase I or early Phase II trials.

## Pre-clinical screening

The rarity of paediatric cancer as well as ethical considerations necessitate that the agents for testing be carefully and rigorously selected, after both *in vitro* and *in vivo* testing to determine activity as well as the optimal schedule. Current NCI guidelines suggest that a drug that is active against multiple cell lines is likely to be active in xenograft models, and if activity shown *in vivo*, to be clinically active. Agents that fail to demonstrate activity in pre-clinical setting are most likely to fail in the clinical setting. Thus, pre-clinical evaluation enables one to exclude agents and combinations of agents that do not demonstrate effectiveness from entering clinical trials. An analysis of the activity of compounds tested in pre-clinical *in vivo* and *in vitro* assays by the NCI’s Developmental Therapeutics Program was reported by Johnson et al. [1] For 39 agents with both xenograft data and Phase II clinical trials results available, *in vivo* activity in a particular histology in a tumor model did not closely correlate with activity in the same human cancer histology, casting doubt on the correspondence of the pre-clinical models to clinical results. However, for compounds with *in*

*in vivo* activity in at least one-third of tested xenograft models, there was correlation with ultimate activity in at least some Phase II trials. Thus, an efficient means of predicting activity *in vivo* models remains desirable for compounds with anti-proliferative activity *in vitro*. Using the hollow fiber assay, there was a higher level of predictivity of *in vivo* xenograft activity. Furthermore, potency in a cell line screen had a high correlation with activity in the hollow fiber assay, both for activity and lack of activity. [1] Biologicals present an additional challenge, as they often do not lend themselves to *in vitro* testing, which is more economical, but must be tested directly in animal models.

### The tumor as a target

Early approaches to specific targeting of solid tumors utilized monoclonal antibodies. Thus far, in paediatrics, the GD2 disialoganglioside was noted to be a favorable target in neuroblastoma. It is highly expressed and not modulated from the cell surface of virtually all neuroblastoma cells, with only weak expression on a restricted range of normal human tissues (particularly peripheral nerves). This antigen has been exploited for imaging and therapy, both directly and as a vehicle for targeted radiotherapy delivery. [2] As a single agent for patients with relapsed disease, modest response rates of 10-15% have been reported. [3-5] More recent attempts to improve these results have included development of a chimeric form of the antibody Ch14.18, and a new humanized form, to reduce the formation of neutralizing antibody (human anti-mouse and human anti-chimeric antibody). Addition of GM-CSF to stimulate ADCC and IL-2 to activate natural killer cells has been tested for feasibility and shown pre-clinical promise. [6,7] Based on promising pre-clinical testing showing ablation of bone marrow and liver metastases in a neuroblastoma model, [8] a Phase I trial of the immunocytokine, Hu14.18-IL2 has recently been completed in the Children's Oncology Group (COG), and a Phase II study is beginning.

Neuroblastoma is a tumor derived from sympathetic nervous system, and therefore expresses high levels of the noradrenalin transporter (NAT), whereby it can internalize and then store catecholamines. [9] Metaiodobenzylguanidine (MIBG) is an analogue of norepinephrine which,

when labeled with radioactive iodine, shows high sensitivity and specificity for imaging neuroblastoma, as well as neuroendocrine tumors. 131I-MIBG has shown excellent activity as a targeted radiotherapeutic, with response rates of near 40% in relapsed patients [10,11] and promising activity in newly diagnosed patients either alone [12] or given with chemotherapy. [13] Unlike some of the other biologicals, a cytotoxic effect on proliferating hematopoietic precursors often results in significant myelosuppression, due to the non-specific radiation to the red marrow. [14,15]

Tumor differentiation is another approach suggested by the propensity of some tumors to undergo spontaneous differentiation and growth arrest. This characteristic was noted in neuroblastoma, and led to Phase I and II trials, [16] followed by a large randomized study in the Children's Cancer Group testing children in a state of minimal residual disease after induction and consolidation therapy. Treatment with 13-cis-retinoic acid in a more dose intensive schedule developed in pre-clinical testing, [17] resulted in effective plasma concentrations and significantly improved event-free survival in high-risk neuroblastoma. [18] Other retinoids, such as fenretinide, may work via other mechanisms, such as ceramide metabolism, [19] and therefore have a broader application in malignancy. [20] Fenretinide has shown promising activity in pre-clinical studies and is currently being evaluated in Phase I and II trials. [21]

Tyrosine kinase inhibitors are under extensive investigation for treatment of cancer, due to the promising results with imatinib targeting of bcr-abl for CML. Since imatinib also inhibits c-kit and PGDFR, it was postulated that it might have activity in other malignancies than just CML. For that reason, Phase I and II trials in paediatric brain tumors have been undertaken, after a few pre-clinical investigations showed potential activity in Ewing's, medulloblastoma, and neuroblastoma. [22-25] The Trk receptors A, B, and C are highly expressed in neural tumors, and a Phase I study of CEP-701, an oral pan-Trk inhibitor is underway in the New Approaches to Neuroblastoma Consortium (NANT), based on *in vivo* studies showing inhibition of tumor growth in xenograft models. [26] EGFR inhibitors, now approved for treating lung and colon cancer, are

also under testing in paediatric cancers. The potential effect of blocking the EGFR pathways is still under pre-clinical investigation in paediatric tumors. [27,28] A number of these that are currently approved for adult epithelial malignancies, including gefitinib, erlotinib, and cetuximab (a monoclonal antibody against the EGFR), are in Phase I testing in paediatric solid tumors in the COG and the Paediatric Brain Tumor Consortium (PBTC). [29] a novel chimeric protein linking the EGFR binding protein, TGF- $\alpha$  to a pseudomonas exotoxin is also in testing in paediatric brain tumors. Her-2/neu is another tyrosine kinase receptor widely under investigation for therapy of epithelial cancers, which may also have some applicability in paediatric osteosarcoma, where expression has been noted, though the significance remains controversial. [30-34] A phase II window trial in newly diagnosed metastatic osteosarcoma for patients whose tumors express Her-2/neu is currently in progress in the COG.

Manipulation of tumors through alteration of gene expression via farnesyl transferase inhibitors, demethylating agents, histone deacetylase inhibitors (HDACI) or downstream alterations in metabolism is another area of interest. The COG and PBTC has had Phase I and II trials of R115777, the farnesyl transferase inhibitor, for leukemia and CNS tumors. Open trials of HDACI in the COG include valproic acid and depsipeptide, while decitabine combined with chemotherapy is being tested in another Phase I study. Agents which alter glutathione content of tumors may enhance sensitivity to alkylating agents. Pre-clinical and clinical studies of such a compound, buthionine sulfoximine, have shown activity in adult cancers and more recently in neuroblastoma. [35,36] Alterations of ceramide metabolism, as discussed above, may also enhance apoptosis, and new drugs which further synergize with fenretinide in this regard are in pre-clinical development. [37]

### **The microenvironment as a target**

The microenvironment also provides an interesting biological approach to treating tumors, as angiogenesis, tissue factors involved in invasion and metastasis such as matrix metalloproteinases, integrins and cytokines, and host immunologic response have all been shown

to play a role in tumor progression. Anti-angiogenic and anti-metastatic agents have been a major focus in recent trials, since it is widely accepted that tumor growth beyond a few cubic millimeters cannot occur without the induction of a new vascular supply. In theory, inhibiting new blood vessel formation should be relatively selective for tumor cells, since endothelium in normal tissue is usually quiescent. Vascular endothelial growth factor (VEGF) is the best-characterized pro-angiogenic factor. In many paediatric tumors, higher levels have been correlated with more aggressive disease. [38,39] Effective blockade of the VEGF pathway has been demonstrated with multiple agents: neutralizing antibody, receptor tyrosine kinase inhibitors (see above), and ribozyme or anti-sense molecules targeting expression. [40-44] Recent studies of the neutralizing antibody bevacizumab, and small molecule tyrosine kinase inhibitor SU5416, demonstrate that, while unlikely to be effective as monotherapy, incorporation of VEGF blockade into cytotoxic regimens may increase overall response rates. However, incorporation may also produce new toxicities, including thromboembolic complications and bleeding, and there may be other effects in young children on growth and development. Current COG and PBTC trials are testing bevacizumab, lenalidomide (a potent immunomodulatory and anti-angiogenic analog of thalidomide), SU5416, as well as tyrosine kinase inhibitors, and the anti-integrin, cilengitide.

Finally, alteration of the host immune response provides another avenue for overcoming resistance, with monoclonal antibodies (see above), cytokines, and various types of vaccines. Interleukin 2 (IL-2) is the most extensively investigated cytokine in clinical use at present. Interleukin-2 enhances the proliferation, cytokine production and cytolytic activity of T and NK/LAK cell populations, various aspects of monocyte/macrophage function and global measures of immune responsiveness in vivo. [45] Although IL-2 has demonstrated pre-clinical anti-tumor activity, it has been disappointing in paediatric clinical trials when used by itself. [46] IL-2 may be more effective in combination with other immunotherapeutic agents, such as the anti-GD2 antibody or other cytokines. [6,8] Recent

preclinical evidence suggests that in combination, IL-12 with IL-2 may possess potent immunomodulatory and anti-tumor activity that exceeds the effect of either agent alone in several murine models. [47,48] Phase I dose escalation trial of IL-12 combined with IL-2 is now underway in the NANT consortium, based on pre-clinical efficacy against murine neuroblastoma. Pre-clinical and clinical trials are also in progress to try to further enhance the specificity and efficacy of cytokines by using autologous tumor cells transfected with cytokines such as IL-2, IL-12, GM-CSF, interferon gamma, or lymphotactin as vaccines to stimulate the host immune response to solid tumors. Other vaccine approaches include the use of DNA vaccines, or dendritic cell vaccines. [49-56]

## Summary

Biological therapy of childhood cancer provides a new approach to overcoming resistance by using agents with a different mechanism of action than standard cytotoxic therapy. Better understanding of the genetic pathways and better pre-clinical models to define effective combinations and schedules for the prioritization of clinical testing will increase the likelihood of fulfilling the promise of targeted therapy.

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# Ethical and philosophical - Challenges of taking the ‘bench to the bedside’

Conrad Fernandez

The study and application of biological agents holds a potential key to many exciting and untapped frontiers in children with malignancy and many other diseases<sup>1-3</sup>. While we are already reaping the benefits of improved diagnosis, surveillance and therapy through this novel field in adults<sup>4</sup> and children<sup>5</sup>, there are many potential ethical challenges that face us, both as researchers at the bench and as clinicians at the bedside. Research in children is a moral responsibility and an absolute key to continuing advancement in health care<sup>6</sup>. This paper examines the principles and regulations that should concern us as researchers in taking “the bench to the bedside” and highlights specifics in biologics research that pose special challenges.

## Definition and Principles

Translational research is defined as clinical research by which knowledge that has been gleaned in a basic science setting (i.e. molecular, genetic, cellular or animal) is translated into diagnostic or therapeutic applications to treat or prevent disease. Typically translational research involves individuals with or at risk for specific diseases. The definition immediately conjures up ethical challenges that must be considered including safety issues, early phase trial designs, surrogate decision makers for incompetent paediatric patients, exploration of assent, justice issues with respect to accessibility and cost and potential conflicts of interest in advancing the agenda of the pharmaceutical industry or individuals versus the needs of the patient. Many of these challenges are overt and easily recognized but some subtle but serious issues require attention to detail and study design to avoid compromising the principle of respect for persons.

The Helsinki Declaration on Ethical principles for Medical Research involving Human Subjects<sup>7</sup>

provides us guidance in outlining the primacy of the patient’s best interests with due attention to maximizing potential benefits whilst minimizing harms. The selected recommendations that are placed at risk of violation in the context of translational research are highlighted in table 1. Broadly, these fall into categories that address respect for autonomy, beneficence, non-maleficence and justice. These are all vulnerable in the field of translational research where firm knowledge is usually absent and rapidly evolving, competing interests may exist within the same individual (hope, altruism, alleviation of suffering), and scientific and societal pressures may cloud one’s judgment.

Translational therapeutic research in paediatric oncology must also be recognized to occur in a complex context. Most patients will be in the circumstance of having exhausted curative standard therapies, most will have a limited ability to assent to participation in research, most will have parents who are torn between balancing family and quality versus quantity of life for the child with cancer, and most will have doctors who are both care-givers and researchers.

Lastly, there is increasing recognition that there is a moral obligation to offer to provide a summary of research results to research participants or their guardians, which they indicate they desire despite potential negative effects<sup>8-10</sup>. Translational research, by their nature, often yields only immature data. This may lead to concern with respect to the reliability and validity of the interpretation of the findings. The advanced stage of illness (and thus high mortality) of the participants complicates the provision of the results to parents of children who took part in phase I studies.

## Regulations

International and national regulatory guidelines have generally incorporated the ethical principles

espoused in the Declaration of Helsinki in codifying requirements for ethical conduct of human research. The Council for International Organizations of Medical Sciences (CIOMS) has published international guidelines for medical research (updated 2002) that indicate how the ethical principles that are set forth in the Declaration of Helsinki can guide the conduct of human research. In particular, these guidelines recognize the unique vulnerability of populations in developing countries and their socioeconomic circumstances and speak to conduct of externally sponsored clinical trials in low resource countries.

In addition to standard research ethics considerations, the policy in the United States under the Title 45 Code of Federal Regulations Part 46. Protection of Human Subjects<sup>11</sup>, Part D affords special protections in research in children. An important standard in this policy is the assessment of risk in the context of potential benefit. Section 46.406 and 46.407 apply most directly to translational research at the phase I level in which the research offers more than minimal risk but potentially no or minimal benefit to the individual child. Similarly, Canadian regulations require careful assessment of risk in determining acceptability of research practices<sup>12</sup>. It should be noted that risk potentially involves more than physical risk in encompassing psychological, emotional, social, and spiritual considerations.

As for all research, it is well accepted that a duly constituted Research Ethics Board/Institutional Review Board must review all translational research for scientific validity and compliance with ethical standards and procedures, before conduct of the proposed study<sup>13</sup>. This is not an easy task and there is some evidence that application of US minimal risk categories by IRB chairpersons is variable and suboptimal<sup>14</sup>. Partially in response to this challenge, the American Society of Clinical Oncology has proposed a central IRB, although the wholehearted acceptance of the notion of a central IRB is variable<sup>15, 16</sup>.

### Phase I studies

Phase I trials have well documented challenges that epitomize some of the ethical concerns that arise in translational research<sup>17</sup>. A commonly

expressed concern is an apparent lack of understanding by both patients and their physicians of the primary aim of phase I trials<sup>18</sup>. Full disclosure, adequate understanding and unrestricted voluntariness are key ethical tenets that may be obscured in consent for phase I studies in advanced cancer patients. However, a recent review suggests that disclosure is usually adequate<sup>19</sup>. The review also demonstrates a more advanced understanding by patients than usually thought. Patients often express a mixture of recognition that they might not benefit, but hope that the therapy will have a positive impact on their own health. Agrawal argues that patients with advanced cancer may have a different set of values and are therefore not coerced by their circumstances<sup>19</sup>.

Recent evidence highlights the changing landscape in this arena of research from primarily cytotoxic chemotherapy to more targeted biological agents, involving a wide array of receptor/ signal transduction, antiangiogenesis, gene transfer and vaccines<sup>20</sup>. Indeed, only one-quarter of phase I CTEP (National Cancer Institute Cancer Therapy Evaluation Program) sponsored trials between 1991 and 2002 were of classical single agent cytotoxic chemotherapy. Horstman suggests that overall response rates have improved (up to 45%) and toxic deaths remain consistently low (<1%). He also points out that phase I trials may confer other benefits that are not traditionally measured (hope, symptom control, quality of life, altruism)<sup>20</sup>. This information is important in the complex process of fully informing parents of the risks and benefits of undertaking phase I research.

Novel biological agents add a further dimension to phase I trial design in that they typically have different end-points and clinical applicability than traditional cytotoxic agents<sup>21</sup>. There may be difficulty in determining the correct biological agent, as is illustrated by anti-angiogenic therapy<sup>22</sup>. Anti-angiogenic compounds and other targeted antitumor agents are more likely to require non-traditional outcomes for phase I trials, such as surrogate markers capable of identifying the therapeutic target and response in the tumor. This is in addition to traditional measures of their pharmacokinetic and pharmacodynamic properties<sup>3</sup>. Biological agents may require non-therapeutic serial biopsy to

determine tumor markers of efficacy; a strategy that adds to cost and fits poorly with maintaining a positive benefit-to-harm ratio and minimization of risk<sup>23</sup>.

Tissue collection in paediatric patients for correlative studies in cancer clinical trials must pose no more than a minor increase in risk over minimum risk and thus creates a special challenge. A CTEP sponsored workshop delineated four recommendations in to consider in study design in order to justify the use of a tissue specimen for correlative studies: **1.** a scientifically or clinically important research question with adequate validity must be posed, **2.** parents must be informed of realistic risks, study procedures and benefits of phase I protocol participation, **3.** research procedures should be clearly defined and separated from clinically necessary ones and, **4.** the correlative component of the study should be voluntary<sup>24</sup>. These recommendations challenge researchers to utilize innovative approaches (surrogate biological markers, imaging techniques and preclinical models) to simultaneously advance the care of these children while acknowledging their special protections afforded in the Code of Federal Regulations 11. Kodish reports that most IRBs process phase I trials with no more scrutiny than others but are likely to have special procedural safe-guards<sup>25</sup>. Consent for phase I studies must encompass these measures of outcome, uncertainty of late effects and novel phase I designs aimed at respecting paediatric needs (i.e. rapid dose escalation, prioritizing agents).<sup>26, 27</sup>

### **Phase II studies**

Wherein cytotoxic agents may be expected to cause radiologically measurable diminution in size of tumors as a primary end-point in phase II trials, some biological agents may be cytostatic. The dependability of the observation of stable disease is more difficult to accept given a commonly observed high degree in variation in rates of progression of human cancers. Thus, a larger number of patients must be exposed to the novel agent in order to demonstrate efficacy. Modifications in trial design (such as randomized discontinuation<sup>28</sup>, single arm – patient as own control<sup>29</sup>) to accommodate for these variations have been suggested and will need to be

clarified for participants in obtaining consent.

### **Gene therapy and genetics**

Many biological agents are likely to exhibit their effect best as a function of long term exposure<sup>30</sup>. Strategies for this include gene therapy in which a host of ethical concerns have been raised related to vector safety, insertion mutagenesis, and therapeutic misconception. Henderson et al carefully articulate the difficulty of obtaining consent for early phase genetic research and the need for expressing unambiguous information while balancing realistic hopes and expectations<sup>31</sup>.

Beyond gene therapy, tremendous implications to individuals, their families and communities may occur with research that studies cancer susceptibility or other genetic characteristics<sup>32, 33</sup>. The American Society of Clinical Oncology Policy Statement on Genetic Testing for Cancer Susceptibility outlines recommendations for clinical practice, research needs, informed consent, protection from discrimination and access to genetic services<sup>34</sup>. With respect to children, ASCO specifically recommends deferring genetic testing unless there are evidence-based risk reduction strategies available or the cancer is expected to predominantly occur in childhood. Researchers, as clinicians, will be faced with an increasing complexity of respecting confidentiality of genetic results while acknowledging “a duty to warn” family members and perhaps community of significant results<sup>35, 36</sup>. Translational research priorities must carefully balance these recommendations against seeking to understand the molecular underpinnings of cancer in childhood.

### **Conflict(s) of interest**

A great deal of attention has been paid to the identification and disclosure of conflict of interest by individuals engaged in research. This attention is rooted in a desire to maintain public and scientific trust in the independent judgment of the researcher and the unbiased expression of the results. Focus has primarily evoked concerns about potential physician/researcher’s financial conflicts in which the well being of research subjects, the good conduct of the trial and the accurate interpretation and reporting of

results may be subsumed to financial gain. Examples of potential difficulties are myriad and ubiquitous (stock holdings or options, consulting income, pharmacy bureau speakers to name a few).

Two models may be utilized to manage financial conflict of interest – a presumption that any financial interest should be prohibited or a model in which there is an allowance of potential conflict alleviated by disclosure and peer review. Unfortunately policies from medical schools and research institutions are inconsistent in the definition and management of financial conflicts of interest. This has led to the questioning of adequate standards for scientific integrity<sup>38, 39</sup>. Potential research recruits indicate that disclosure of financial conflict by the researcher is strongly valued as part of the informed consent process<sup>40</sup>.

Conflict of interest problems may extend beyond financial concerns and, indeed, the individual researcher. Researchers in academia face constant pressure to produce research results and compete for scarce research funding. Their career advancement of stature and tenure is linked to productivity. These more subtle conflicts of interest inevitably shape the research agenda of individual researchers.

Institutions also increasingly face difficulties in balancing financially driven industry relationships against academic values encompassing freedom to publish and carry out independent research agendas, including moral obligations to address developing world priorities<sup>41</sup>. The ever increasing proportion of industry-provided financial investment in academic research (62% in 2000) is certainly linked to reporting of bias manifest in pro-industry conclusions when industry sponsorship is present<sup>42, 43</sup>. At least part of this bias (Odds ratio 3.6) has been ascribed to flawed trial designs that favor positive results and publication bias in suppression or delay of negative or neutral results<sup>43</sup>. Company formation by academic researchers may lead to profound conflicts of interest related to commitment, intellectual property rights and exploitation of university resources<sup>44</sup>. The university itself becomes exposed to conflict in taking on equity ownership of companies. Institutional strategies to mitigate these conflicts include strict and high

standards of disclosure, physical separation of facilities, restrictions on interactions between investment and research staff, and oversight by independent bodies (with lay public representation)<sup>45</sup>

### Justice

Increasingly attention is being paid to issues of justice – the ethical obligation to treat each person in accordance with what is morally right and proper, to give each person what is due to him or her. In the ethics of research involving human subjects the principle refers primarily to *distributive justice*, which requires the equitable distribution of both the burdens and the benefits within populations participating in research<sup>46</sup> (i.e. not using third world subjects solely as a means to an end). Justice also includes reasonable access to agents that have been shown to be effective as investigational or interventional tools (a direct challenge in translational research and an increasing problem for agents that have extremely high development and commercial utilization costs<sup>47</sup>). Each of these must be addressed in maximizing respect for children.

While not unique to paediatric translation research, Sung et al have identified two major obstacles to translation of research findings to the bed side - first at the step of basic science to human studies and second from human studies to clinical practice<sup>48</sup>. They identify many contributing factors that could be targets for remediation including inadequate recruitment of participants, regulatory burdens<sup>49</sup>, fragmented infrastructure, training and inadequate mentoring and financial support of clinical investigators, and informatics<sup>48</sup>. These are justice issues in establishing societal priorities in support human health and well-being.

### Summary

Research in children is a moral responsibility. The translational researcher and clinician face difficult ethical challenges in such a vulnerable population. Attention to individual and institutional conduct will be critical as this area evolves an ever-increasing complexity of methodologies and strategies of research conduct.

**Table 1. Selected recommendations (with article reference number) from the Declaration of Helsinki that are at risk for violation in the conduct of translational research.**

Declaration of Helsinki Recommendations	Ethical principles at increased risk of violation in translational research (reason[s])
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.	<b>Beneficence</b> (dying patients may be used as a means to an end)
8. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.	<b>Justice</b> (developing nations may) <b>Autonomy</b> (difficulty in determining valid consent and assent in children)
12. Appropriate caution must be exercised in the conduct of research, which may affect the environment, and the welfare of animals used for research must be respected.	<b>Justice</b> (exploitation of limited/ vulnerable resources)
13. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.	<b>Respect for persons</b> <b>Conflicts of interest</b> (financial, promotional, stature, institutional pressure)
17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed.	<b>Non-maleficence</b> (necessary limited knowledge in phase I studies)
18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject.	<b>Beneficence</b> (Phase I studies may offer little direct benefit)
22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail.	<b>Autonomy</b> (difficulty in explaining complex study designs in vulnerable population under duress from advanced cancer)
23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.	<b>Autonomy</b> <b>Beneficence</b> (clinician often has dual role as care giver and researcher)

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

### Autonomy

Respect for persons

(Assent is a complex area that is difficult to assess)

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

### Justice

(limited resources of individual or community to continue to receive novel and costly interventions)

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— SECTION **B** —

# Ewing Tumours: Management and Prognosis

Michael Paulussen, Sir Alan W. Craft

## Case Description

A 15 year old boy complains of pain in his right thigh. Pain is independent of activity and sometimes even occurs at night. Physical examination shows a slight swelling. Radiographs demonstrate an osteolytic lesion in the femoral diaphysis, MRI shows a medullary tumour with penetration of the cortex and surrounding soft tissue involvement. Chest CT scan, whole body technetium bone scintigraphy, and iliac crest bone marrow biopsy exclude metastases. Diagnostic biopsy of the femoral lesion shows a malignant (grade III), small-round-blue cell tumour, immunohistochemistry (CD99 positivity) and molecular biology (translocation t(11;22)(q24;q12)) confirm the diagnosis of a Ewing tumour. He is started on alkylator- and doxorubicin- based treatment in the framework of a multinational trial (Euro-E.W.I.NG. 99). After six courses, his tumour is completely resected, an endoprosthesis is implanted. After an additional 8 courses of chemotherapy he finishes treatment and enters long term follow-up.

## Epidemiology

Ewing tumours (ET) are the second most common primary bone malignancies in childhood and adolescence with an annual incidence of 3 per million in the Caucasian population, the male:female ratio is 1.5:1. The median age at presentation is 15 years, but ET are observed in all age groups. Pelvic bones, the femur, the thoracic skeleton, tibia, fibula and spine are the bones most commonly affected, but other sites including purely soft tissue tumours do occur. 25% of cases present with metastases at diagnosis in lungs, bones or bone marrow, other metastatic sites like lymph nodes, central nervous system or liver are very uncommon.<sup>[1,2]</sup>

## Diagnosis

Pain is the presenting symptom in most cases, later followed by tenderness, swelling, and loss of function. As most patients are in the second decade of life, sport injuries are often recalled and may falsely delay proper diagnostic measures. Thus, pain lasting for more than 4 weeks, or pain independent of activity, should prompt imaging studies even if a “trauma” is recalled. The conventional radiograph demonstrates an aggressive osteolytic lesion, typically penetrating the periosteum, leading to “spiculae” and “onion skin” phenomena. Magnetic resonance imaging (MRI) reveals a medullary tumour with an often massive extraosseous soft tissue mass. Imaging must include the whole involved bone with both adjacent joint in order to detect “skip metastases”. Screening for metastases must include chest computed tomography (CT) and whole body technetium bone scan – or, where available, 16-fluorodesoxyglucose positron emission tomography (16-FDG PET), as well as bone marrow aspirate and biopsy (taken at a site distant from the primary tumour, e.g. iliac crest). The definitive diagnosis must be made as soon as possible by biopsy. The biopsy should be performed by a team experienced in the management of malignant bone tumours, as the biopsy channel has to be regarded as tumour contaminated and must later on be included in the definitive local treatment. The biopsy specimen must be saved both as fixed tissue and as fresh (frozen) material in order to allow for sufficient diagnostic testing. This should be performed by an experienced bone pathologist and molecular biologist: Small, blue, round tumour cells are found at light microscopy, CD 99 (mic-2 antigen) is commonly positive. Definitive diagnosis today includes RT-PCR demonstration of a rearrangement of the EWS (Ewing’s sarcoma) gene on chromosome 22,

most often as translocation t(11;22)(q24;q12). This genetic alteration is found both in Ewing's sarcomas and the so-called malignant peripheral neuroectodermal tumours (PNET) including Askin tumours of the chest wall. Today, all these tumours are denoted "Ewing tumours" or "Ewing family of tumours". [3-9]

As both the correct choice of surgical approach, and proper preparation and processing of the biopsy specimen are demanding, the patient should ideally be transferred to a specialised centre for the biopsy procedure.

## Treatment

### Chemotherapy

In 1921, James Ewing realised these tumours were radio-responsive. However, all of his first series of patients died within two years from distant metastases. [10] This situation changed only when in the 1970s the application of cytostatic drugs in addition to local therapy was introduced. Since the early studies of Rosen and others [11-13], three to five drug regimens based on alkylating agents like cyclophosphamide (CYC) or ifosfamide (IFO), doxorubicin (DOX), vincristine (VCR), topoisomerase inhibitors like etoposide (ETO), and actinomycin D (ACT) have become standard. In the IESS studies, it could be demonstrated, that the addition of DOX significantly enhanced the efficacy of a VCR, ACT, CYC schedule. [14,15] The second COG-CCG study indicated that the addition of IFO and ETO to the above mentioned four drugs increased survival in paediatric patients with localised disease. Most important, all patients with this rare disease should be treated in the existing large intergroup trials, e.g. in the Euro-E.W.I.N.G. 99 study framework. In this European-American cooperative project, as an example of a modern ET therapy, all patients – paediatric or adult – receive six course of VCR, IFO, DOX, ETO (VIDE) followed by local therapy – wherever feasible complete surgery – and further consolidation therapy. This consolidation therapy is stratified and randomised in four treatment arms, depending on initial stage of disease, histological response, and local therapy applied. With this trial, two conventional regimens are compared for standard risk patients, while for high-risk patients conventional treatment is compared to high-dose therapy with stem cell rescue. [17]

### Local therapy

There has been a long debate over the optimum choice of local therapy: Surgery or radiotherapy. Nowadays, surgery is recommended wherever feasible, with radiotherapy applied to patients in whom surgery is not feasible, or where surgery was incomplete, and/or where in the surgical specimen, a poor response to induction chemotherapy is demonstrated. [18]

### Prognostic Factors

The most important predictor of outcome is the stage of disease at diagnosis: In localised disease, 10-year survival is about 65%, while in patients with metastases at diagnosis, only 15-35% survival can be achieved, depending on the sites involved – patients with lung metastases fare better than those with bone, bone marrow or other metastases. Second to "stage", the histological response to induction chemotherapy is of most significance in predicting outcome, followed by other less indicative factors like tumour size, or patient age. Of note, patients who cannot undergo surgery are at a higher risk of local relapse and of a fatal outcome. [19]

### Outlook

The unique feature of a defined chromosomal rearrangement leading to a specific tumour has initiated efforts to identify potential therapeutic targets, but so far no major breakthrough has been achieved. For the time being, optimising therapeutic schedules and drug combinations, and testing of newer drugs in vitro and possibly in vivo are the next steps to take. Moreover, tailoring treatment to individual prognostic features in order to avoid both over- and under-treatment is a matter of research in current trials.

### Conclusion

Early and appropriate use of imaging techniques, immediate patient transfer to an experienced bone tumour centre, proper biopsy, and qualified histopathological and molecular biological diagnosis are the most important steps to early diagnosis. Once the diagnosis of a Ewing tumour is established, patients with this rare disease should be treated within cooperative clinical trials in order to define risk pattern, tailor treatment to individual risks, optimise treatment schedules, and test new drugs for their

therapeutic potential. Moreover, large trial frameworks assure optimal and qualified treatment and help to avoid undue adverse effects of treatment. When these aspects are observed, the majority of Ewing tumour patients may be cured today.

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# Non-Hodgkin's Lymphoma In Children And Adolescents

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## Introduction/Epidemiology

Sixty percent of all childhood lymphomas have been classified as non-Hodgkin's lymphoma, representing 3% of all childhood malignancies for children younger than 5 years, and 8-9% for children and adolescents 5-19 years of age. In the US, 750-800 cases of non-Hodgkin's lymphoma are diagnosed annually in children and young adults younger than 20 years of age.<sup>(1-4)</sup> There is also an increased incidence of non-Hodgkin's lymphomas, especially B-cell lymphomas, in children with inherited and/or acquired immunodeficiencies.

The vast majority of childhood non-Hodgkin's lymphomas are high-grade tumors with aggressive clinical behavior. Unlike adult non-Hodgkin's lymphoma that is predominantly B-cell phenotype, paediatric lymphomas are almost equally divided between B- and T-cell neoplasms, and follicular or low grade lymphomas are distinctly uncommon.<sup>(5-8)</sup> There

are four major subtypes of childhood non-Hodgkin's lymphoma: Burkitt lymphoma (classic and atypical Burkitt lymphoma), lymphoblastic lymphoma, diffuse large B-cell lymphoma (DLBCL) and anaplastic large cell lymphoma (ALCL). The distribution of these four main histologic subtypes includes approximately 40% Burkitt lymphoma, 30% lymphoblastic lymphoma, 20% diffuse large B-cell, and 10% anaplastic large cell lymphoma.

Additionally, correct staging is critically important at the time of diagnosis since most children present with advanced disease at diagnosis. The St. Jude's Children's Research Hospital staging classification, modified from the Ann Arbor system for Hodgkin's disease, took into consideration the common presentations of childhood non-Hodgkin's lymphoma including increased extranodal involvement, metastatic spread to the bone marrow and CNS, and noncontinuous spread of disease (Table 1A).<sup>(9)</sup>

**Table 1A : St. Jude's Staging Classification for Childhood Non-Hodgkin's Lymphoma**

<p><b>Stage I</b></p> <p>A single tumor (extranodal) or single anatomic area (nodal) with the exclusion of mediastinum or abdomen</p> <hr/> <p><b>Stage II</b></p> <p>A single tumor (extranodal) with regional node involvement</p> <p>Two or more nodal areas on the same side of the diaphragm</p> <p>Two single (extranodal) tumors with or without regional node on the same side of the diaphragm</p> <p>A primary gastrointestinal tract tumor with or without mesenteric nodes, grossly and completely excised</p>	<p><b>Stage III</b></p> <p>Two single tumors (extranodal) on opposite sides of the diaphragm</p> <p>Two or more nodal areas above and below the diaphragm</p> <p>All of the primary intrathoracic tumors (mediastinal, pleural, thymic)</p> <p>All extensive primary intra-abdominal disease</p> <p>All paraspinal or epidural tumors, regardless of the other tumor site(s)</p> <hr/> <p><b>Stage IV</b></p> <p>Any of the above with initial CNS and/or bone marrow involvement (&lt;25% malignant cells)</p>
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*From Murphy SB N Engl J Med 1978;299:1446.*

Limited-stage disease (stage I and II) is defined as one or two masses on one side of the diaphragm, whereas more advanced disease (stages III and IV) has been defined as either metastatic disease including the CNS and/or bone marrow disease or disease on both sides of the diaphragm and extensive intrathoracic and intra-abdominal disease. However, the St. Jude's staging classification is unclear on the definition of extensive primary disease and considers all primary abdominal and thoracic tumors as extensive stage III disease, despite original surgical debulking, which may occur at diagnosis.<sup>(9)</sup> Subsequently, a new French, American, and British (FAB) staging classification was developed (Table 1B) that better defines the staging of childhood B-large cell and Burkitt lymphoma of childhood.<sup>(10)</sup> This staging classification was applied in the DLBCL and Burkitt lymphoma international study (LMB-FAB) within the Children's Cancer Group (CCG), United Kingdom Children's Cancer Study Group (UKCCSG), and the Societe Francaise d'Oncologie Pediatrique (SFOP) study groups.<sup>(10)</sup>

**Table 1B : FAB\* Staging System for Childhood B-Large and Burkitt Lymphoma**

<p><b>Group A</b></p> <p>Completely resected Stage I (St. Jude)</p> <p>Completely resected abdominal Stage II (St. Jude)</p>
<p><b>Group B</b></p> <p>All patients not eligible for Group A or Group C</p>
<p><b>Group C</b></p> <p>Any CNS involvement† and/or bone marrow involvement (&gt;25% blasts)</p>
<p>*FAB: French, American, British</p> <p>†CNS: Any L3 blast, cranial nerve palsy or compression, intracerebral mass, and/or parameningeal compression</p> <p>From Cairo et al, Med Pediatr Oncol 1997;29:320a</p>

## Diffuse Large B-Cell Lymphoma (DLBCL)

### Treatment and prognosis of Limited Stage Childhood and Adolescent Diffuse Large B-Cell Lymphoma (DLBCL)

Children and adolescents with limited disease DLBCL, either St. Jude's stage I and II, CCG limited stage, or FAB group A, have an excellent prognosis with an estimated 5-year event-free survival (EFS) of 90-95%. Over the past 10-15 years, there has been significant progress in reducing the amount of therapy required for limited stage childhood DLBCL and eliminating the need for radiotherapy. While surgery plays a major role in the diagnosis and/or complete resection of limited DLBCL, multiagent chemotherapy in large part accounts for the excellent survival recently reported. The length of treatment for childhood limited stage DLBCL currently ranges between 6 weeks to 6 months of multiagent chemotherapy. Current chemotherapy regimens that have been successfully utilized in this clinical setting include COMP (CCG and POG) (3-6 months), COPAD (x 6 weeks) (FAB), or cyclophosphamide and prednisone (CP) followed by dexamethasone/ifosfamide/Ara-C/VP-16/methotrexate and dexamethasone/cyclophosphamide/methotrexate/doxorubicin (12 weeks) (BFM).<sup>(11-16)</sup>

### Treatment and Prognosis of Childhood and Adolescent Advanced DLBCL

The prognosis of advanced childhood and adolescent DLBCL has improved significantly over the past decade.<sup>(12,15,17-20)</sup> The chemotherapy combinations that have been used for advanced large B-cell (non-anaplastic) childhood lymphoma include "APO" + Ara-C/VP-16 (POG), "Orange" (CCG), LMB (FAB) and BFM-NHL (BFM).<sup>(12,15,17-20)</sup> The recent use, however, of short but intense chemotherapy such as FAB/LMB 96 (FAB), Orange (CCG) or BFM NHL90 (BFM) has now resulted in a greater than 90% 3-year survival rate in children with advanced B-large cell (non-anaplastic) lymphoma.<sup>(12,15,20,21)</sup> The CCG hybrid regimen "Orange", which consists of CHOP-based induction, VP-16/ifosfamide intensification and DECAL intensification and a similar maintenance phase with a slight decrease in intensity, results in a 90% overall survival rate in children and adolescents with advanced

DLBCL.<sup>(19,22)</sup> Similarly, Patte et al, utilizing an FAB/LMB-type regimen of COP reduction, COPADM intensification, and CYM consolidation in children and adolescents with advanced DLBCL, demonstrated a 90% 5-year EFS.<sup>(12,21)</sup> Reiter et al, utilizing a BFM approach of CP, ifosfamide, methotrexate, dexamethasone, Ara-C, VP-16 and cytarabine/methotrexate/cyclophosphamide/dexamethasone/doxorubicin, demonstrated a 95% 3-year EFS.<sup>(15)</sup> All three of the above advanced B-LCL approaches have utilized a chemotherapy regimen designed to treat Burkitt (classic and atypical) lymphoma. Laver et al, however, utilizing an approach more designed for LCL and not Burkitt lymphoma (APO + VP-16/Ara-C), only demonstrated a 78% EFS with this approach.<sup>(18)</sup> Patients with advanced DLBCL that still have a somewhat inferior outcome include those with a primary mediastinal DLBCL and patients with bulky tumors (stage III) with elevated LDH levels.<sup>(21)</sup>

## **Burkitt Lymphoma in Children and Adolescents**

### **Treatment and Prognosis in Limited Stage Childhood and Adolescent Burkitt Lymphoma (BL)**

Similar to limited stage DLBCL, children and adolescents with limited stage, either St. Jude stage I and stage II, CCG limited stage, or FAB group A, with Burkitt lymphoma have a superb prognosis with an estimated five-year EFS of 90-95%. Similar to limited stage childhood DLBCL, the requirement for radiotherapy has been eliminated over the past decade as a requirement for treatment in children with limited stage Burkitt lymphoma. With minimal chemotherapy (range 6 weeks to 6 months), the prognosis is excellent, ranging between 90-95% five-year EFS.<sup>(11-16)</sup> There are several multiagent chemotherapy regimens that have been utilized by a variety of paediatric cooperative groups that have resulted in this excellent outcome, including COPAD (6 weeks) (FAB), COMP (3-6 months) (CCG and POG), or cyclophosphamide and prednisone (CP) followed by dexamethasone/ifosfamide/Ara-C/VP-16/methotrexate and dexamethasone/cyclophosphamide/methotrexate/doxorubicin (BFM) (12 weeks).<sup>(11-16)</sup>

### **Treatment and Prognosis of Childhood and Adolescent Advanced Burkitt Lymphoma**

The most dramatic advances in the cure of childhood non-Hodgkin's lymphoma have been the significant improvement in disease-free and overall survival of advanced Burkitt lymphoma over the past twenty years. In four consecutive Children's Cancer Group (CCG) studies from 1977 through 1995, there has been a steady improvement in the 3-year disease-free survival in children and adolescents with advanced Burkitt lymphoma.<sup>(23)</sup> Patte et al, utilizing an LMB-type regimen of COP reduction, COPADM intensification, and CYVE consolidation in children and adolescents with advanced Burkitt lymphoma, recently demonstrated a 90% 3-year disease-free survival.<sup>(12)</sup> Reiter et al, utilizing a BFM approach (BFM95) (R2 and R3) regimens in children with advanced Burkitt lymphoma, reported an estimated 4-year event-free survival of 89% and 74% for bulky disease B-NHL and B-ALL, respectively.<sup>(15)</sup> Most recently, the international FAB/LMB 96 study has demonstrated that with standard FAB therapy, children and adolescents with bone marrow involvement have a >90% 3-year EFS and patients with CNS involvement have a 71% 3-year EFS with short and intensive chemotherapy.<sup>(24)</sup>

Children and adolescents with bone marrow and/or CNS involvement have an inferior outcome if they have a non response to reduction chemotherapy with COP or have combined bone marrow and CNS disease.<sup>(24)</sup> Although elevated LDH is still associated with being a poor-risk factor for both stage III BL and DLBCL, LDH has not been shown to be prognostically important in patients with BM and/or CNS disease. Furthermore, in the most recent FAB/LMB 96 and BFM NHL 95 studies, both studies have demonstrated that cranial irradiation can be eliminated in patients with CNS positive disease with the substitution of more aggressive high-dose methotrexate and additional intrathecal chemotherapy.<sup>(24,25)</sup>

### **Lymphoblastic Lymphoma (LBL)**

Encompassing 20-25% of childhood NHL, LBL is morphologically indistinguishable from acute lymphoblastic leukemia (ALL). Approximately

80% of LBL are of immature T cell phenotype and 15-20% precursor B lineage. T-LBL most commonly presents with advanced stage disease, while precursor-B LBL more often presents with localized disease and may present at unusual sites such as skin or bone<sup>(26)</sup> or with disease below the diaphragm.

T-LBL is derived from thymic T cells, expressing the pan-T antigen CD7. Other antigens which may be expressed, depending on the stage of differentiation, are CD2, CD3, CD45RO, as well as other markers of immature T cells such as CD4 and CD8 double-positivity or double-negativity.<sup>(5)</sup> Precursor B-cell LL expresses the childhood ALL phenotype (CD10, CD19, CD22, HLA-DR). Many different translocations may be seen in T-LL, usually involving translocation of a protooncogene to one of the T-cell receptor (TCR) genes, a/d on chromosome 14q11.2, less commonly b on chromosome 7q35 or TCR gamma 7p14-p15, resulting in aberrant expression of the oncogene. The most common translocations are t(11;14) (p13;q11) in 7%, t(10;14)(q24;q11) in 5% and t(1;14)(p32-p34;q11) in 3% of cases.<sup>(27,28)</sup> Other common abnormalities involve the *tal-1* gene on chromosome 1, found to be overexpressed in 30% of T-leukemia/lymphoma.<sup>(28)</sup> Genetic alterations that result in *tal-1* activation or novel TAL-1 protein interactions are seen in the majority of T-cell ALL and NHL.<sup>(27)</sup> Finally, inactivation of the multitumor suppression gene (*mts-1*), located on chromosome 9p21, may be the most common genetic defect found in T-cell leukemia /lymphoma.<sup>(27)</sup>

## Therapy of T-Lymphoblastic lymphoma

### Localized LBL

Induction therapy results in a 95% CR rate in the 15% of early stage NHL patients with localized LBL, however, this subgroup is prone to late relapses. In the POG 9219 trial, with 9 weeks induction therapy and 6 months of continuation therapy (6-mercaptopurine [6-MP] and low dose methotrexate [MTX]), the EFS was only 60%, the majority of patients relapsed in the bone marrow. Most were salvaged, giving an overall survival (OS) of >90% at 5 years.<sup>(29)</sup> In contrast, the BFM group used more intensive therapy and achieved an EFS of 90% using the standard arm of the BFM T-cell protocol<sup>(30)</sup> (standard BFM induction, consolidation phase consisting of 4 doses of high dose [5 gm/m<sup>2</sup>] MTX and maintenance therapy given for 2 years). No reinduction therapy or local or cranial radiation was given for stage I and II patients. A recently-opened COG study will attempt to confirm the BFM results. If successful, this strategy will increase the risk of therapy-related morbidity, but will obviate the need for intensive salvage therapy for 40% of the patients with localized LL.

### Advanced stage LBL

Advanced stage LBL has been shown to respond best to protocols designed for acute lymphoblastic leukemia (ALL). Local radiation therapy, although effective, results in significant late risks particularly, when applied to the mediastinum, and is unnecessary if adequate chemotherapy is given.<sup>(31)</sup> The results of the various chemotherapeutic regimens are shown in Table 2.

**Table 2 : Outcome for Patients with Lymphoblastic Lymphoma**

Protocol	Number	Stage	EFS (%)	Follow-up (mo)	Reference
LSA2L2 /ADCOMP	281		74 64	60	(37)
POG 8704	218	III/IV	67	60	(32)
LMT 81	76 8	III/IV I/II	76 73	57	(38)
UKCCSG	59	III/IV	65	60	(36)
BFM90	82 19	III IV	90+3 95+5	60	(30)

Results of POG 8704 in which both T-ALL and T-LBL patients, randomized to receive L'asparaginase 25,000 u /m<sup>2</sup> weekly x20 during maintenance, did significantly better than the no-L'asparaginase group<sup>(32)</sup>, as well as preliminary results of the BFM-95<sup>(33)</sup> study suggest that this drug is important in therapy of T-cell disease. In the BFM studies -86, -90 and -95, the results were better for males, with an EFS of 87% compared to 77% in females. The worst prognosis was seen in adolescent females, with a pEFS of 51% ± 19%.<sup>(34)</sup> Other risk factors for failure were evaluated and only the presence of "B" symptoms was found to be prognostic (EFS of 74% vs 87% in patients without symptoms). Despite an earlier study in which incomplete tumor resolution at day 60 of therapy produced a RR for relapse of 3.55<sup>(35)</sup>, incomplete resolution at day 33 or the end of induction did not predict for failure in the BFM-90 study.<sup>(33)</sup> Reiter also evaluated the need for cranial irradiation (CRT). 357 patients received CRT, 52 (15%) relapsed of whom 8 (2%) had CNS involvement<sup>(33,36)</sup>, compared to 410 patients without CRT, 118 of whom relapsed, 18 (4%) with CNS disease.<sup>(30,36-38)</sup> In CCG-502 CRT was eliminated, with an incidence of isolated CNS relapse of only 2%, and the SFOP study also suggested that CRT can successfully be replaced by intensive chemotherapy including high dose MTX. The open COG study and BFM-95, do not give CRT for CNS-negative T-LBL patients, but the studies are being closely watched. Finally most studies gave 24 months of therapy for T-LBL but in the BFM-90 study all relapses occurred by 12 months from diagnosis<sup>19</sup>, suggesting that therapy duration can safely be shortened to 18 months.

Thus the cooperative group studies suggest that ALL type therapy is effective for T-LBL. It appears that L'asparaginase is an important drug, and several, but not all, studies also suggest that high dose MTX results in a survival advantage. The studies further suggest that dose intensity in the first 4 weeks of therapy may be important<sup>(33)</sup> and that cranial radiation may not be necessary if either high dose MTX and/or intensive intrathecal therapy is given. Similarly local radiation therapy is not indicated if adequate chemotherapy is given. The SFOP and BFM studies suggest that even in patients with testicular disease at diagnosis, testicular radiation is only indicated

for residual disease after high-dose MTX. It appears that more than 50% of the relapses are local, often mediastinal and that salvage after relapse is poor.

### B-Precursor LBL

Approximately 20% of LBL express B-cell markers. Nuclear TdT+ and lack of surface Ig expression are decisive parameters in differentiating B-LBL from mature B-cell neoplasms.<sup>(39)</sup> Because of small patient numbers, the correct treatment for B-lineage LL has not been clearly defined. Twenty-seven children with precursor B-cell LL were treated on the BFM 86 and BFM 90 NHL trials, 21 on ALL-type therapy with 2/21 relapses, 6 on Burkitt-type therapy with 3 of 6 relapsing including 2 with localized disease<sup>(40)</sup>; all 3 were salvaged with ALL-type therapy, suggesting that patients with B-lineage LL should be treated as ALL for a therapy duration of 18-24 months. The pEFS for the total group was 0.73 (SE 0.10) and pOS 92% (SE 0.05) at 10 years<sup>(40)</sup> correlating well with the results of a recent review which found 98 reported patients, 64% <18 years old.<sup>(39)</sup> Approximately 75% had skin disease (with or without adjacent nodal disease), lymph node, bone, head and neck, and retroperitoneum. Mediastinal disease was uncommon. Five patients (4.8%) had CNS disease. Eighty-one patients had long term follow up data, 60/81 (74%) are disease free (median. 28 months).<sup>(39)</sup> Thus with ALL type therapy, the majority of patients have a good outcome.

### Anaplastic Large Cell Lymphoma (ALCL)

ALCL represents 10%-15% of paediatric NHL. Approximately 65% of ALCL are of T cell phenotype and 35% null cell.<sup>(41)</sup> B-cell lymphomas with anaplastic histology have been moved to the category of diffuse large B-cell lymphomas.<sup>(41,42)</sup> ALCL cells react with antibody to CD30, epithelial membrane antigen (EMA) is generally positive, and CD25 (IL2-R) is often expressed, other markers are variable. T-ALCL cells usually express an aberrant T-cell phenotype, (usually activated helper T-cell phenotype (CD4+), lacking one or more pan-T cell antigens, usually CD3.<sup>(41)</sup> Clusterin, a highly conserved glycoprotein, is found in the majority of ALCL, allowing differentiation from Hodgkins disease.<sup>(41,42)</sup> The majority of childhood ALCL

show a characteristic translocation t(2;5)(p23;q35) relocating a promoter sequence of the nucleolar phosphoprotein (*npm*) encoding gene on 5q35 to the anaplastic lymphoma kinase gene, *alk* on 2p23, resulting in production of the fusion protein ALK, a tyrosine kinase. Pleiotrophin (PTN), the ligand that activates ALK, has been shown to have a role in tumorigenesis in nude mice.<sup>(43)</sup> ALK can be recognised immunohistochemically on fixed tissue, using an anti-ALK1 monoclonal antibody. NPM-ALK is found in ~80% of ALK pos ALCL, the remainder being due to variant translocations; at least 8 have been described to date. NPM-ALK has a characteristic nuclear and cytoplasmic distribution pattern, thus the variant ALK fusion proteins, most of which are cytoplasmic in distribution, can be easily identified. A minority of primary cutaneous ALCL (PCALCL) and Hodgkins (HL) show NPM-ALK transcripts but no detectable protein, thus detection of ALK reliably differentiates ALK+ ALCL from other CD30+ tumors such as LYP, HL and PCALCL.<sup>(42)</sup> Survivin, a target of the STAT3 pathway, is activated in approximately 50% of ALCL and may predict unfavorable prognosis, independent of ALK status, at least in adult patients.<sup>(44,45)</sup> ALCL commonly involves unusual sites such as bone, skin and peripheral nodes and may present with manifestations such as diffuse pulmonary disease not common in other lymphomas. It may present with a systemic or primarily cutaneous

distribution. PCALCL is more indolent than systemic ALCL and has a better prognosis. Despite the often-widespread nature of ALCL, involvement of CNS and marrow are uncommon.<sup>(46)</sup>

### Therapy of Systemic ALCL

For advanced-stage ALCL, the major study groups have used very different strategies, varying from short-pulse chemotherapy (BFM), to more prolonged chemotherapy derived from T-cell protocols (SFOP, CCG), to inclusion on protocols designed for all large cell lymphomas (POG). The results of the various protocols are shown in Table 3.

Duration of therapy for advanced ALCL varied from 4-5 months (BFM-90) to 7-8 months (SFOP) to 12 months (POG9315). In the BFM studies, relapses tended to occur with a mean time of 8 months after achieving remission.<sup>(47)</sup> Le Deley evaluated risk factors for ALCL in the combined BFM, SFOP and UKCCSG studies and found that in multivariate analysis of 235 patients with a median follow up of 47 months, mediastinal involvement ( $p=0.004$ ), lung, spleen and/or hepatic disease ( $p=0.006$ ) and skin lesions ( $p=0.02$ ) were associated with a significantly poorer outcome.<sup>(48)</sup> Based on this, two risk groups were delineated: standard (EFS 87%, OS 92%), and high risk (skin, mediastinal and/or visceral disease) (EFS 61%, OS 67%).

**Table 3 : Treatment Outcome for Patients with ALCL**

Protocol	Number	Stage	EFS (%)	Med Follow-up (Mo)	Reference
BFM 90	8	I	100	30	(47)
	20	II	79+9		
	55	III	74+6		
	5	IV	50+20		
SFOP-LM 89, 91	82	I/II III/IV	94 55	49	(49)
LSA2L2/LSA4	19	III/IV	56	60	(50)
UKCCSG	72	III/IV	59	51	(51)
CCG-5941	86	III/IV	78+5	16	(52)
POG 9315	86	III/IV	71.8+6	48	(53)

In summary therefore, the cooperative trials suggest that children with T-cell/null-cell ALCL can be successfully treated with strategies varying from short-pulse intensive chemotherapy to longer less intensive, non-alkylator protocols. CNS and bone marrow involvement is unusual in ALCL and intermediate- or high-dose MTX with (BFM) or without intrathecal therapy (SFOP) or even IT therapy alone (COG) effectively prevents CNS relapse without CRT. Risk factors for relapse include the presence of mediastinal, visceral and/or skin involvement and possibly "B" symptoms. Current study group protocols are investigating the efficacy of the addition of weekly vinblastine to standard chemotherapy for ALCL, based on the successful SFOP salvage therapy results.

### Summary

The prognosis for children and adolescents with non-Hodgkin's lymphoma, both with limited and advanced stage disease, has improved significantly over the past two decades. Except for rare subtypes, the chance of being alive and disease free at five years for limited and advanced stage disease B-NHL is 95% and 80%, respectively. The prognosis for advanced lymphoblastic non-Hodgkin's lymphoma in children and adolescents has now increased to over 85% survival. The prognosis, however, for the most advanced childhood and adolescent ALCL is still less than 70% at 7-year follow-up. The improved outlook for childhood non-Hodgkin's lymphoma, however, has not come without a certain price. The use of short but intense chemotherapy, especially in B-NHL, has resulted in long hospitalizations and severe hematopoietic and non-hematopoietic toxicity. Furthermore, long-term complications or late effects, such as sterility, cardiomyopathy, and secondary malignancies, still occur following the use of the aggressive multiagent chemotherapy in children and adolescents with advanced non-Hodgkin's lymphoma.

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# Risk-based Treatment for Children with Neuroblastoma

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## Neuroblastoma Tumors are Heterogeneous

The clinical hallmark of NB is heterogeneity, with the likelihood of tumor progression varying widely according to age at diagnosis, the stage of disease, and tumor biology<sup>(1,2)</sup>. Numerous transformation-linked genetic and epigenetic changes have been identified that have contributed to the understanding of neuroblastoma tumor biology, and many of these tumor-specific genetic and epigenetic aberrations are highly predictive of treatment response and outcome<sup>(1,3,4)</sup>. Because of the heterogeneous behavior of neuroblastoma tumors, modern treatment strategies are stratified according to patient risk, defined by both clinical and biological factors. Numerous studies have validated the concept of risk group-directed therapy and the usefulness of biological prognostic variables. However, at the present time, the risk-grouping systems used in North America, Europe, Japan, and Australia are not uniform. It is, therefore, difficult to directly compare the results of clinical trials conducted in different regions. To address this problem, efforts to develop a uniform International Risk Group (INRG) classification system are ongoing. Below we have outlined the criteria that are currently used to define risk group and stratify treatment within COG and the European SIOP Neuroblastoma Group (ESIOP NB).

## Patient Age and Stage

As with most malignancies, stage of disease is a significant prognostic factor in NB<sup>(5)</sup>. Over the past two decades, several different staging systems have been used to classify extent of disease. The Evans staging system<sup>(6)</sup> is based on clinical criteria, whereas both the Paediatric Oncology Group (POG)<sup>(7)</sup> and the tumor-node-metastasis (TMN) classifications<sup>(8)</sup> are surgicopathologic systems. In an effort to develop a uniform staging system based on both clinical and surgicopathologic findings, the INSS

was developed by an international consensus group<sup>(9,10)</sup>. Retrospective analyses have confirmed that the INSS criteria identify prognostic subsets of patients with NB<sup>(11,12)</sup>, and the INSS has now been implemented world wide.

Age at diagnosis remains the only other independent clinical prognostic factor. For all stages of disease beyond localized tumors, infants less than 1 year of age have significantly better disease-free survival than older children with equivalent stages of disease.<sup>(6,13)</sup> The currently accepted age cut-off of 365 days was based on the observations made by Breslow and McCann over 30 years ago<sup>(13)</sup>. Although their results suggested that days of age should be used for risk stratification as a continuous variable, this is not clinically practical. Instead, age has been used as a prognostic variable with the convenient cut-off of 365 days. More recent studies suggest that 365 days may not be optimal age to discriminate risk. Look and co-workers reported that 1- to 2-year old children with disseminated disease have a better outcome than children over 2 years of age<sup>(14)</sup>. Two additional studies have demonstrated excellent outcome in a subset of toddlers (12-18 months of age) with stage 4 disease who were treated with multi-agent chemotherapy<sup>(15,16)</sup>.

In an effort to identify the statistically optimal age cut-off, London and co-workers recently analyzed the influence of age on outcome in 3,666 patients treated on CCG and POG studies<sup>(17)</sup>. These studies confirmed that the prognostic contribution of age to outcome in neuroblastoma is continuous in nature. Although no clear delineation of an age cut-off was detected, strong statistical evidence was found for an age cut-off between 15-19 months. Further analyses of a larger, international cohort of patients are ongoing. Based on these studies, it is anticipated that a new age cut-off for risk-group assignment will be incorporated into the INRG.

### MYCN Amplification

MYCN amplification occurs in approximately 20% of primary NB tumors and is strongly associated with the presence of metastatic disease and poor prognosis<sup>(18)</sup>. These observations suggest that *MYCN* critically contributes to the clinically aggressive behavior of high-risk NB tumors, and a number of laboratory studies support this hypothesis. The level of expression of *MYCN* has been shown to directly correlate with growth potential of NB cells<sup>(19,20)</sup>. More recent studies have shown that *in vivo* tumor growth can be inhibited with *MYCN* antisense oligomers<sup>(21)</sup>. Transgenic mice with targeted expression of *MYCN* have provided further evidence of the critical role *MYCN* plays in NB pathogenesis<sup>(22)</sup>.

### COG Risk-Group Classification System and Treatment Stratification

In addition to age (< vs > 1 year), INSS stage, and tumor *MYCN* status, the current COG Risk Classification System also includes tumor histology and ploidy. Treatment is stratified according to risk-group assignment as indicated in the Table below.

The overall objective of the recently closed COG low-risk study (P9641) was to preserve the excellent survival rate for patients with low-risk NB by using surgery as the primary treatment approach, thereby minimizing the risks of acute and long-term chemotherapy-related morbidity for the majority of these patients. Based on the excellent outcome and high rate of spontaneous remission of localized tumors that has been reported in the mass screening studies<sup>(23)</sup>, the COG has also recently developed a clinical trial in which infants with small adrenal primary NBs will be observed rather than undergo major surgery (ANBL00P2).

Excellent survival rates have also been observed for patients with intermediate-risk disease following treatment with chemotherapy and surgery<sup>(24,25)</sup>. However, the use of adjuvant therapy for patients with regional disease has been challenged in a single institution study in which a subset of patients with INSS stages 2B and 3 tumors that lacked *MYCN*-amplification survived without disease progression following surgery alone<sup>(26)</sup>. These observations suggest that for the majority of patients with biologically favorable regional tumors, chemotherapy may

**Table : COG Risk-Group Classification System**

INSS Stage	Age	MYCN Status	Shimada	DNA Index	Risk Group
1	0-21 y	Any	Any	Any	Low
2A/2B	< 365 days	Any	Any	Any	Low
	> 365 days-21 years	Normal	Any	-	Low
	> 365 days-21 years	Amplified	Favorable	-	Low
	> 365 days-21 years	Amplified	Unfavorable	-	High
3	< 365 days	Normal	Any	Any	Intermediate
	< 365 days	Amplified	Any	Any	High
	> 365 days-21 years	Normal	Favorable	-	Intermediate
	> 365 days-21 years	Normal	Unfavorable	-	High
	> 365 days-21 years	Amplified	Any	-	High
4	< 365 days	Normal	Any	Any	Intermediate
	< 365 days	Amplified	Any	-	High
	> 365 days-21 years	Any	Any	-	High
4S	< 365 days	Normal	Favorable	> 1	Low
	< 365 days	Normal	Any	= 1	Intermediate
	< 365 days	Normal	Unfavorable	Any	High
	< 365 days	Amplified	Any	Any	High

be safely reduced or eliminated. In an effort to avoid associated acute and long-term complications while maintaining high cure rates, adjuvant chemotherapy and radiotherapy have been reduced in the current COG Intermediate-Risk Study (A3961). Intermediate-risk patients with favorable biology tumors are treated with a short course of chemotherapy (4 cycles), while intermediate-risk patients with unfavorable biology receive a longer course of chemotherapy (8 cycles).

Unfortunately, outcome remains poor for children with high-risk disease. During the past decade there has been only a modest improvement in survival, which is thought to be due to intensification of induction chemotherapy, megatherapy consolidation, and improved supportive care. Several clinical trials, including the large prospective randomized CCG-3891 study which demonstrated superior outcome for patients randomized to myeloablative therapy and bone marrow transplant versus chemotherapy during consolidation<sup>(27)</sup>, support the hypothesis that dose intensification is an important component of successful treatment of NB<sup>(28)</sup>. The differentiation agent 13-cis retinoic acid was shown to be clinically effective when administered in the setting of minimal residual disease in the randomized CCG 3891 clinical trial<sup>(27)</sup>. This seminal study demonstrated that a biologic agent was capable of impacting outcome in high-risk NB.

The COG is currently conducting a study that includes 6 cycles of intensive induction chemotherapy and surgery, followed by myeloablative chemotherapy with PBSC rescue and local radiation (A3973). Patients are randomized prospectively to receive purged PBSCs versus non-purged PBSCs. Patients who achieve a VGPR/CR are then eligible for a second randomized study testing the efficacy of Ch14:18 anti-GD2 antibody plus cytokines in combination with 13-cis retinoic acid to 13-cis retinoic acid alone in the setting of minimal residual disease. It is anticipated that this study will be closed in accrual in the Spring of 2006.

### **ESIOP NB Risk-Group Classification System and Treatment Stratification**

The European SIOPEX Neuroblastoma Group (ESIOP NB) was founded in 1994. Its first study

(L NESG 1), which was activated on January 1995, was designed for patients with localized disease (approximately half of all NB patients). Three subgroups were defined based on the extent of the initial surgical approach (complete tumor resection, resection with minimal residue, resection with gross residue or biopsy only). In an effort to define common criteria for tumor operability, surgical risk factors (SRF) were identified based on the radiological characteristics of the tumor. In presence of any SRF, the surgeon was encouraged to limit the primary operation to a biopsy and perform a second surgery following chemotherapy. As a consequence, some patients who would previously have been considered operable were entitled to receive neo-adjuvant chemotherapy and were defined stage 2 unresectable (2U). Preliminary results of the study indicate that patients who underwent surgery in presence of SRF had lower chance of having a complete tumor resection and had a greater risk of developing surgery-related complications.

L NESG 1 included a trial for patients with stage 2 disease without *MYCN* gene amplification for whom no adjuvant therapy was to be given. Of 124 evaluable patients with a median follow up of 62 months, OS at 5-year is 93.0% and RFS is 83.0%.<sup>29</sup> The occurrence of relapse was greater in presence of unfavorable histology (INPC categories<sup>30</sup>) and high LDH level.

A second study (IN ES Study), which was designed for infants with NB, opened in 1999. It included four trials:

- 99.1 was developed for infants with unresectable disease. These patients received low-dose chemotherapy (Vincristine plus Cytosine, or Carboplatin plus Etoposide in case of symptomatic spinal cord compression, or other relevant symptoms);
- 99.2 was developed for infants with stage 4s (independently of primary tumor extension). These patients were observed if the Philadelphia score was under 2 for infants greater than 1 month of age or if the score was 1 for younger age infants. Patients with stage 4 disease without bone, lung, or CNS metastases were also eligible for this trial in the absence of relevant clinical symptoms. Bone disease was defined as positive skeletal

mIBG uptake with abnormal X-ray and/or CT findings

- 99.3 was designed for patients with stage 4 with bone disease (positive skeletal mIBG uptake associated with radiological or CT abnormalities), or metastases to lung, or CNS;
- 99.4 was developed for patients with stage 2, 3, 4 and 4s with documented *MYCN* gene amplification in tumor cells.

The four trials were closed to patient registration in June, 2004. Preliminary results after a median follow-up of two years are as follows:

- 99.1 of 110 evaluable patients, 10 relapsed (8 local, 2 metastatic) after a median of 16 months from diagnosis. OS and EFS are respectively 100% and 91%
- 99.2 of 104 evaluable patients, 52 did not receive any chemotherapy. There were two disease-related deaths with OS 97.3% and EFS 89.6%
- 99.3 of 48 evaluable patients, 4 relapsed with one subsequent disease-related death. OS and EFS are 97.7% and 85.9%, respectively

99.4 of 42 evaluable patients, none died of toxicity. Thirty % of patients did not respond or experience disease progression during treatment. Only 14 underwent megatherapy. Overall results were poor (OS and EFS 36%), and were worse for patients with stage 4 compared to other stages.

In February 2002, ESIOP NB activated a Protocol for high-risk patients including stage 4 above 1 year and stage 2 and 3 with amplified *MYCN* gene. The therapeutic scheme included induction therapy with COJEC regimen (8 cycles given at 10-days intervals), followed by high-dose chemotherapy (randomization between CEM and BuMel) in case of response, irradiation of primary tumor site and oral retinoic acid. The protocol is on-going and has recruited approximately 500 patients so far.

The ESIOP NB Risk-Group Classification System is shown in the Table below. Similar to COG, treatment is stratified according to risk-group assignment.

**Table 2 : ESIOP NB Risk Group classification**

ESIOP Stage	Age	<i>MYCN</i> Status	Risk Group	Protocol	Chemotherapy
1	0-18 y	Any	Low	LNESG 2	None
2R & 3R	0-11 m 0-11 m >12 m – 18 y >12 m – 18 y	Normal Amplified Normal Amplified	Low High Low High	LNESG 2 99.4 LNESG 2 HR-NBL-1	None Intensive None Very intensive
2 U & 3 U	0-11 m 0-11 m >12 m – 18 y >12 m – 18 y	Normal Amplified Normal Amplified	Intermediate High Intermediate High	99.1 99.4 Unresectable HR-NBL-1	Low-dose Intensive Low-dose Very intensive
4	0-11 m s bone mets ^ 0-11 m c bone mets 0-11 m >12 m – 18 y	Normal Normal Amplified Any	Low Intermediate High High	99.2 99.3 99.4 HR-NBL-1	None* Low-dose Intensive Very intensive
4S	0-11 m 0-11 m	Normal Amplified	Low High	99.3 99.4	None* Intensive

R, resectable; U, unresectable; s=without; c=with

^ Bone metastases are defined as mIBG positive spots associated with abnormal X-ray and/or CT findings.

\* in absence of life-threatening symptoms

## Future Directions

Although substantial progress has been made in the treatment of children with low- and intermediate-risk NB, cure rates for high-risk patients remain low. Additional dose-escalation of therapy for high-risk patients is likely to be prohibitive. Furthermore, despite this aggressive treatment approach, more than 50% of children with high-risk disease will relapse due to drug-resistant residual disease. Eradication of refractory microscopic disease remains one of the most significant challenges in the treatment of high-risk NB. Phase I and II studies testing new targeted therapies are being conducted throughout the world, and preliminary results suggest that targeted radiotherapy, immunotherapeutic molecules, new retinoids, anti-angiogenic agents, and other experimental therapeutics have activity against refractory disease. A number of studies integrating biologically-based treatment approaches with cytotoxic treatment are ongoing or in various phases of development. Hopefully, this approach will lead to improved survival for children with high-risk NB.

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# The role of chemotherapy in medulloblastoma

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**D**uring the last 20 years, pilot studies and randomized trials have been performed in medulloblastoma. Indications of chemotherapy can now be given according to the extension of the disease at diagnosis and also according to the age of the patient.

## Average risk medulloblastoma patients

The SIOP III trial compared EFS for patients with an average risk medulloblastoma treated with standard (36-56 grays) craniospinal irradiation alone and patients who received vincristine, carboplatin, cyclophosphamide and etoposide before standard craniospinal irradiation. The EFS probability at 5 years for the group that received chemotherapy and radiotherapy was 73% whereas that for the group that received only radiotherapy was 60% ( $p=0.04$ )

However, it is admitted that in average risk medulloblastoma patients the dose of irradiation can be reduced at 23.4 grays to the craniospinal axis and 54 grays to the posterior fossa when effective chemotherapy is delivered. A pilot study was performed, delivering weekly vincristine concurrent to reduced-dose irradiation with eight cycles of adjuvant chemotherapy consisting of lomustine, cisplatin, and vincristine delivered after the completion of irradiation. The PFS of 65 patients aged 3 to 10 years was 79% at 5 years. The French M-SFOP 93 study delivered two courses of 8 in 1 chemotherapy and two courses of etoposide and carboplatin before reduced-dose craniospinal irradiation. The 5-year RFS of the 136 included patients was 64% (+/-8%).

The treatment related late complications remain of major concern in these patients and the current SIOP IV study compares the early and late toxicity of hyperfractionated radiotherapy with that of conventional irradiation ; in both arms the patients receive a chemotherapy consisting of lomustine, cisplatin, and vincristine.

## High risk medulloblastoma patients

Historically high-risk medulloblastoma patients treated with craniospinal radiation therapy alone had a 5-year PFS of 20-40%. A variety of chemotherapy regimens have been attempted to improve this outcome.

Fifteen patients with M+ disease treated with radiotherapy and chemotherapy consisting of lomustine, cisplatin, and vincristine had a 5-year PFS probability of 67% (+/-15%). In a CCSG study 188 high risk medulloblastoma patients were randomly assigned to receive either 8/1 chemotherapy before and after standard dose craniospinal irradiation or weekly vincristine during craniospinal irradiation followed by eight cycles of lomustine, vincristine, prednisone. The 5-year PFS of the entire cohort was 54%. It was 63% for the group of patients treated with lomustine, vincristine and prednisone.

In a POG study 94 metastatic medulloblastoma patients were randomly assigned to receive three cycles of cisplatin and etoposide before or after irradiation; all patients subsequently received eight cycles of cyclophosphamide and vincristine. The 5-year EFS was 65%. At St Jude four courses of high dose cyclophosphamide, cisplatin and vincristine followed with stem cell rescue have been delivered after craniospinal irradiation. The 4-year EFS for high risk patients was 74%.

It is likely that more intensive chemotherapy can improve the prognosis of high risk medulloblastoma patients. In the mentioned studies patients with isolated postoperative local residual or patients with M1, M2, M3 stages are not always clearly identified. Furthermore in these studies the dose of irradiation has often been increased and it is difficult to know the respective rôle of chemotherapy and irradiation.

Further investigative studies are mandatory.

## Young children

Sequelae from tumor and its treatment, especially craniospinal irradiation, are more severe in young children. For these reasons, different approaches have been explored using prolonged postoperative chemotherapy in order to delay or avoid irradiation in this age group.

In the Baby POG1 protocol, 62 children less than 3 years of age receive postoperative chemotherapy and delayed craniospinal irradiation after the age of 3. The 5-year PFS and OS are 31% and 39% respectively.

It is now clear that the initial extension of the disease is a very strong prognostic factor when the place of radiation therapy is reduced.

It has been shown that irradiation can be avoided in patients who had a complete surgical resection and no metastases. In these new approaches the role of salvage treatment for progressing patients before irradiation is important and in the French study the place of high dose busulfan and thiotepa with stem cell rescue followed by an irradiation restricted to the posterior fossa has been developed. In this study, patients who have a surgical complete resection has a 41% 5-year EFS and a 79% 5-year OS.

For patients with metastatic disease at diagnosis investigative protocols are currently evaluated.

The evaluation of the long term sequelae in these patients is mandatory as they may develop complications related to these new approaches, for instance long term complications of high dose agents or leukoencephalopathies due to intra ventricular methotrexate as it is used in the German protocol.

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# Early Response And Minimal Residual Disease Testing In Childhood ALL: Methodologies And Clinical Application

Stephen P. Hunger, Andrea Biondi

## The clinical utility of early response measures

Approximately 80% of children with acute lymphoblastic leukemia (ALL) are cured with contemporary risk-directed treatment, with therapies of different intensities administered to patient groups with differential risks of relapse. Many different clinical and biological features are used to identify different risk groups. Early response to therapy, defined as the initial degree and rate of disease regression prior to end induction, is one of the most powerful. Simple enumeration of blasts remaining in peripheral blood (PB) or bone marrow (BM) at defined times during induction therapy is highly predictive of treatment outcome among large groups of patients with ALL.<sup>(1)</sup>

The Berlin-Frankfurt-Munster (BFM) group showed in ALL-BFM 83 that the number of blasts remaining in the peripheral blood following seven days treatment with prednisone and a single dose of intrathecal methotrexate separates patients into two subgroups with divergent outcomes.<sup>(2)</sup> **Prednisone good responders (PGR) consist of the approximately 90% of patients that have < 1000 blasts/ml remaining in the PB and have an excellent outcome. In contrast, prednisone poor responders (PPR) that have = 1000 blasts/ml PB have an extremely poor outcome. Prednisone response has been a cornerstone of risk stratification in BFM and most other European ALL cooperative group clinical trials for the past several decades.**

In parallel, Children's Cancer Group (CCG) studies conducted in the US and Canada used BM morphology to assess early response and showed significantly worse outcomes for patients with an M3 marrow (= 25% blasts) versus an M1 (<5% blasts) or M2 (5-25% blasts) marrow at day 7 or 14 of induction therapy.<sup>(3,4)</sup> The CCG also showed that early response could

be used to target new therapeutic strategies to subgroups of patients at high risk of relapse. CCG 1882 randomized high risk ALL patients (age = 10 years and/or initial white blood cell count = 50,000/microliter) that had a day 7 M3 marrow to receive either augmented or standard therapy and found that augmented therapy led to major improvements in treatment outcome.<sup>(5)</sup> Once a therapy is proven safe and effective in a high-risk subgroup, it can then be tested in patients with a lower risk of relapse. Pursuing this strategy, the CCG tested augmented therapy in high risk ALL patients with a day 7 M1 or M2 marrow in CCG 1961 and found it to be significantly more effective than standard therapy.<sup>(6)</sup> Similar augmented therapy is now being tested in a large subset of standard risk ALL patients in the Children's Oncology Group (COG) AALL0331 trial.

While poor early response as assessed by PB or BM morphology following 1-2 weeks of therapy can identify patient subgroups at significantly increased risk of relapse, this approach has significant limitations. Many patients with good responses relapse, while many of those with poor responses are cured. Indeed, about 70% of relapses in BFM trials occur in PGR patients.<sup>(7)</sup> Over the past 15-20 years, more sensitive molecular biology and flow cytometric technologies have been developed to assess early response by measuring subclinical levels of minimal residual disease (MRD). To date, modest sized, mostly retrospective, studies have demonstrated that BM MRD measurement in the first 1-3 months of therapy may separate patients into very good and very poor risk subsets better than conventional measures of early response. Studies now in progress will help to define whether or not MRD assessment leads to major improvements in risk stratification and treatment allocation in large clinical trials.

Clinical features (age, white blood cell count), genetic properties of the leukemia cells (presence/absence of specific translocations or chromosome gains/losses) and the host (polymorphisms in key genes) also have major impact on outcome. A major challenge is to define how early response should be integrated with other risk factors to develop the most robust treatment assignment algorithm. The fact that MRD is predictive of outcome in multivariate analyses establishes that it is not simply a surrogate for other prognostic factors.

### Methods for detection of MRD in ALL

Two different technological approaches have been used to measure MRD in ALL: molecular methods that utilize the polymerase chain reaction (PCR) to amplify and quantitate tumor-specific or tumor-associated genetic markers, and quantification of residual leukemia cells with tumor-specific or tumor-associated phenotypes by flow cytometry. Each has relative advantages and disadvantages. Molecular methods generally have greater sensitivity, while flow-based methods are faster and less expensive. Studies have shown consistently that end induction MRD burden correlates with outcome in childhood ALL; the higher the MRD level, the worse the outcome.<sup>(8-12)</sup>

PCR can be used to measure MRD in several different ways. The immunoglobulin (Ig) heavy and light chain and T cell receptor (TCR) genes, collectively termed antigen receptor (AgRec) genes, undergo ordered recombination during B- and T-cell development thereby creating patient-specific markers of the malignant clone. Using AgRec targets that include the Ig heavy chain (IgH), TCR-delta, TCR-gamma and Ig-kappa genes at least one AgRec PCR target can be identified in 98% and at least two AgRec PCR targets in 95% of childhood B-precursor ALLs.<sup>(13)</sup> In most cases, MRD at levels of at least  $10^{-4}$  (1 cell in 10,000), and often  $10^{-5}$ , can be detected using AgRec PCR. A second approach for molecular MRD detection is amplification of fusion RNA transcripts generated by leukemia-specific chromosome translocations via reverse-transcriptase PCR (RT-PCR). About 30-35 % of childhood ALLs contain *BCR-ABL*, *MLL-AF4*, *E2A-PBX1*, or *TEL-AML1* fusion transcripts. Because these transcripts are leukemia-specific,

they clearly distinguish malignant from normal cells. Real time quantitative PCR (RQ-PCR) methods can be used to detect and quantify these transcripts with high sensitivity and reliability.<sup>(14)</sup> However, because they are only present in subsets of patients, it is difficult to use them in large scale clinical trials, unless the trials are focused on distinct molecular subsets of ALL that receive unique therapies, such as Philadelphia chromosome-positive ALL.

Detection of MRD via flow cytometry is based on the principle that leukemia cells express patterns of antigens that differ from those of normal cells.<sup>(15)</sup> These difference are due to leukemia cells expressing novel combinations of antigens that are not encountered during normal lymphoid differentiation or expressing normal antigens in intensity patterns not observed during normal maturation.<sup>(16)</sup> Normal precursor B-cells express a precise and reproducible sequence of antigens during maturation. Using a modest number of 3- or 4-color combinations, leukemia cells can be found to occupy regions of so-called "empty space" on bivariate displays, where normal B cell precursors are not observed.<sup>(17)</sup> Practically speaking, flow cytometry can detect leukemia cells at a sensitivity in the range of  $10^{-4}$  at end induction, and cooperative group experience finds that over 95% of B-precursor ALLs have informative phenotypes.<sup>(17-19)</sup>

### Use of MRD measures in cooperative group clinical trials

North American and European cooperative groups have used very different strategies to integrate MRD measures into risk stratification algorithms in contemporary ALL clinical trials. In Europe, the joint Italian AEIOP, German and Austrian BFM group ALL-BFM-2000 trial uses MRD determined by AgRec PCR at end induction and end consolidation therapy to adjust treatment intensity.<sup>(20)</sup> A substantial amount of work was required to standardize MRD testing at several different laboratories in the three countries, as pioneered by the BIOMED initiative.<sup>(21)</sup> While this study is still accruing patients, it has established that it is feasible to integrate molecular MRD into large-scale cooperative group clinical trials. Over 80% of patients have been risk-stratified on the basis of

MRD using AgRec targets with a sensitivity of at least  $10^{-4}$ . Several different therapy questions are being tested in this trial.<sup>(20)</sup> Standard risk patients are defined as those with a PGR, no t(4;11), t(9;22) or hypodiploidy, and negative MRD (with a sensitivity of at least  $10^{-4}$ ) at end induction and end consolidation. Patients in this group, which includes about 30% of childhood ALL patients, are randomized to receive standard BFM therapy versus a reduced treatment regimen that decreases anthracycline dose in phase IIa (delayed intensification), with a goal of maintaining EFS at levels seen in the BFM 95 trial. At the other end of the spectrum, poor risk patients include those, regardless of initial presenting characteristics, that have MRD  $>1\%$  at end consolidation. Those patients receive either a very intensive chemotherapy regimen or allogeneic stem cell transplant in an attempt to improve what is predicted to be a less than 50% cure rate.

The North American cooperative groups have also used MRD for risk stratification, but with a very different approach. The Paediatric Oncology Group (POG) established in the recently completed to accrual POG 9900 trial that MRD could be determined at end induction via flow cytometry in over 95% of patients. The new generation of COG ALL clinical trials use flow cytometry to assess MRD at end induction, with a primary goal of identifying additional high risk patients for treatment intensification. Patients are assigned to risk groups primarily on the basis of age and initial white blood cell count, genetic features of the leukemia cells, and conventional day 8/15 measures of early response. However, those with end induction MRD burdens of  $= 0.1\%$  are non-randomly assigned to receive more intensive therapy. In addition, a separate low risk group is identified that includes NCI standard risk ALL patients with favorable cytogenetics (*TEL-AML1* fusion or simultaneous trisomies of chromosomes 4, 10 and 17), no CNS or testicular disease, and an excellent early response defined by  $<5\%$  marrow blasts by day 15 and MRD  $< 0.1\%$  at the end of induction. These children will be randomized to receive a “minimal” standard therapy with or without four additional doses of PEG Asparaginase in the first 12 weeks following induction, with a goal of increasing the 6-year EFS from 90% to 94%.

## Summary and perspective

Thus, studies currently in progress will define how useful it is to integrate MRD into ALL clinical trials and will assess the relative advantages and disadvantages of different technical approaches to MRD detection. Major contemporary challenges in the treatment of children with ALL are to identify those patients with an extremely high likelihood of being cured with currently available therapies so that adverse effects of further treatment intensification can be avoided, to identify patients unlikely to be cured with currently available therapies so that they can receive more intensive or novel therapies, and a need to develop better tools with which to measure treatment response to novel molecularly targeted therapies. Accurate measures of early treatment response will play a critical role in addressing each of these challenges.

However, we must also emphasize that one of the most noteworthy developments in contemporary paediatric oncology is organized efforts of resource rich countries to work with resource poor countries to develop systems to provide effective treatment for malignancies such as ALL. We must not lose sight of the fact that simple early response measures that can be performed anywhere, prednisone response and/or assessment of early marrow response, can provide critical information that can be used effectively to stratify therapy intensity based on risk of relapse. Risk stratified therapy may have particularly large benefits in this setting. Toxic death rates from intensive therapy are far higher than observed in resource rich countries, necessitating that these therapies be used cautiously and in a targeted manner. We must not establish a paradigm that suggests that only those centers that can measure MRD can provide effective contemporary therapy.

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# Osteosarcoma

**Mark L. Bernsein, Ian Lewis**

## Introduction

Osteosarcoma is the most frequent primary cancer of bone, curable in about two-thirds of patients with initially localized disease using a combination of surgical excision and adjuvant chemotherapy. Patients with initially metastatic or recurrent disease have less favorable outcomes. Particularly for those populations, therapeutic innovations are required.

## Epidemiology

It is the typical solid malignancy of adolescence: More than 5% of all cancers in the 15 to 19 year age group are osteosarcomas. The approximate annual incidence of osteosarcoma is 2-3/million in the general population. Its peak incidence is in whom it accounts for more than 5% of all cancers. A second, smaller age peak in older patients is due to osteosarcomas arising in abnormal bones, such as those affected by Paget's disease or prior irradiation. Tumors of older individuals will not be discussed further here.

Osteosarcoma affects males approximately 1.4 times more often than females. The primary tumor is usually located in the metaphysis of a long bone of an extremity, with the distal femur and the proximal tibia being the most frequent sites of involvement, followed by the proximal humerus and proximal fibula. Overall, two thirds of all paediatric osteosarcomas arise around the knee. Tumors of the axial skeleton or craniofacial bones occur most frequently in older patients.

## Histology

By definition, osteosarcoma is a mesenchymal malignancy in which the malignant cell population produces osteoid. The extent of osteoid production can vary considerably. Conventional osteosarcoma, a high grade central malignancy of bone, accounts for 80-90% of all osteosarcomas. Its most frequent subtypes

are osteoblastic, chondroblastic, and fibroblastic osteosarcoma. Conventional osteosarcomas, teleangiectatic, high-grade surface and small-cell osteosarcomas all have a very similar clinical course and must be treated by multimodal regimens which include chemotherapy. Low-grade central and parosteal osteosarcomas are treated by surgery only. Craniofacial osteosarcomas, apart from those of the skull, metastasize less frequently than conventional osteosarcomas, as do periosteal osteosarcomas. There is no general consensus as to whether they should be treated by surgery alone or by surgery plus chemotherapy.

## Etiology

The etiology of osteosarcoma remains obscure in most patients. Trauma has often been accused, but little evidence exists to support a causal relationship. The predilection of osteosarcoma for the age of the pubertal growth spurt and the sites of maximum growth suggest a correlation with rapid bone growth. A minority of osteosarcomas are caused by radiation exposure, with the risk related to the dose administered. Exposure to alkylating agents may also contribute. Together, radiation therapy, alkylators, and genetic tumor predisposition, described below, make osteosarcoma one of the most frequent secondary solid malignancies following therapy.

The incidence of osteosarcoma is increased in several well defined hereditary disorders associated with germ-line alterations of tumor suppressor genes, but even taken together, these account for only a few percent of all osteosarcomas. Survivors of hereditary retinoblastoma with germline mutations of the retinoblastoma gene RB1 on chromosome 13q14 carry a risk which is 500 to 1000 times greater than that of the general population. The Li-Fraumeni-cancer family syndrome, in which

germline mutations in the p53 gene are found, is associated with a 15-fold increase. Among patients with sporadic osteosarcoma, approximately three percent will have germline p53 mutations. Although germline mutations of p53 and RB are rare, these genes are altered in many osteosarcoma tumor samples. Consequently, loss of function of the p53 and RB tumor suppressor genes, which regulate cell cycle progression in normal cells, are believed to have an important role in osteosarcoma tumorigenesis. Rothmund-Thomson syndrome and Bloom syndrome, rare conditions caused by mutations in tumor suppressor genes coding for helicases, are also associated with an increase in osteosarcomas, as is Werner syndrome (adult progeria).

Numerous oncogenes are also altered in osteosarcoma tumor cells, but it is not clear which of these events occurs first and why or how it occurs. Moreover, it is not clear which, if any, of the alterations is essential to tumor development and might therefore represent a therapeutic target.

### **Signs, symptoms, and natural history**

Patients with osteosarcoma usually do not feel ill until very late in the course of the disease. They typically seek medical attention because of first intermittent and then continuous pain, often erroneously attributed to recent trauma, for example, a sports injury. Tumor related swelling and loss of function of the adjacent joints usually develop later. In approximately 10%, the first sign of disease is a pathological fracture. Pain at bony sites other than the primary may represent metastatic involvement. Metastases, however, are most likely to occur in the lungs. These produce respiratory symptoms only with extensive involvement. Systemic symptoms, such as fever and weight loss, occur rarely in the absence of very advanced disease.

The differential diagnosis of osteosarcoma includes traumatic lesions, osteomyelitis, benign bone tumors such as exostosis, fibroma, osteoid-osteoma, chondroma, giant-cell tumor of bone, bone cysts, and others, as well as other primary malignant lesions of bone such as Ewing sarcoma or lymphoma, and metastases from malignancies such as neuroblastoma or soft tissue sarcoma.

At diagnosis, metastases are present in 10-20% of patients. Primary metastases are limited to the lungs in 80% of affected individuals. The remainder have bone metastases with or without additional pulmonary involvement. Skip metastases, isolated tumor foci within the same bone as the primary tumor, occur in a minority of patients. Regional lymph node metastases are rare and other sites are almost never involved at initial diagnosis. If no systemic treatment is given, most patients with seemingly localized disease will go on to develop metastases within one to two years. Lungs and, to a lesser extent, distant bones are again the organs involved. Death is usually due to respiratory failure caused by extensive pulmonary involvement.

## **Diagnostic evaluation**

### **History and Physical**

The evaluation of suspected osteosarcoma begins with a detailed history, physical examination, and plain radiographs. As stated above, most patients present with a history of pain of the involved region. Physical examination is typically remarkable only for a mass at the primary site. Loss of motion of neighboring joints, infiltration of the skin, and neurological deficits may occur, depending on the location and extent of the tumor.

### **Laboratory studies**

There are no known specific laboratory parameters. Increases of alkaline phosphatase or lactic dehydrogenase (LDH) serum levels, which are observed in a considerable number of patients, do not correlate reliably with disease extent but may have negative prognostic significance.

### **Imaging**

The characteristics and extent of the primary tumor must be evaluated by plain radiographs and by cross sectional imaging techniques. On plain radiographs, osteosarcoma often presents with lytic or sclerotic changes, or both. Ossification in the soft tissue in a radial or "sunburst" pattern is typical, but neither sensitive nor specific. Periosteal new bone formation with lifting of the cortex leads to the appearance of a Codman's triangle. Magnetic resonance imaging (MRI) is the most useful tool to define the

intramedullary tumor extent, soft tissue component, and relation of the tumor to vessels and nerves.

More than 95% of metastases occur in the lungs or the skeleton. Plain X-rays and a CT-scan of the thorax, preferably high resolution spiral CT, are used to rule out pulmonary metastases. Bone metastases are searched for by a  $^{99m}\text{Tc}$ -MDP bone scan. Skip metastases may also be visualized on the bone scan. MRI of the whole bone is preferable because of its higher sensitivity. There is currently no established role for positron emission tomography (PET), which is inferior to CT for the detection of lung metastases and inferior to bone scintigraphy for the detection of bone metastases. Whole body MRI may lead to a higher detection rate of bone metastases, but its place in diagnostic evaluation remains to be defined.

### **Biopsy**

While imaging will often result in a high index of suspicion, the diagnosis of osteosarcoma must always be verified histologically. It is strongly recommended that biopsies be performed only in specialized centers. Open biopsy may be most suitable to obtain sufficient material for histological evaluation and ancillary studies. The biopsy specimen should be sent to the pathologist without prior fixation.

### **Treatment strategy**

Many patients with osteosarcoma can be cured. Inappropriate use of diagnostic tools and suboptimal initial therapy can irrevocably compromise a patient's chances. Therefore, all patients with osteosarcoma should be treated in specialized, experienced centers able to provide access to the full diagnostic and therapeutic spectrum. Treatment within prospective clinical trials is considered standard clinical practice in many countries.

Local treatment of osteosarcoma is surgical. Most patients, however, have already developed micrometastatic disease by the time their osteosarcoma is detected. Prior to the 1970's, when treatment was exclusively surgical, the outcome was extremely poor. Almost 90% of patients who presented with apparently localized disease developed recurrences and died within one to two years from diagnosis. This dismal

outlook was dramatically improved when multiagent chemotherapy was added to surgery.

### **Effective agents**

The majority of current treatment protocols are based upon two or more of only four active agents, namely doxorubicin, cisplatin, high-dose methotrexate, and ifosfamide.

**Doxorubicin** was first introduced into osteosarcoma treatment in the 1970s and has remained an integral component. Because of the risk of long-term anthracycline cardiotoxicity, osteosarcoma protocols often include cardioprotectants such as dexrazoxane or the reduction of anthracycline peak levels by continuous doxorubicin infusions.

**Methotrexate**, a folate antagonist, blocks the action of dihydrofolate reductase, the enzyme responsible for reducing folate to its active form, tetrahydrofolic acid. In osteosarcoma, methotrexate is given at supralethal doses, usually in the range of 8 to 12g/m<sup>2</sup>. The antidote leucovorin (activated folate) is then administered to bypass the blocked enzyme, the assumption being that normal cells can be rescued more effectively than tumor cells, which may lack active folate transporters. In addition, hydration, alkalinization of the urine, and leucovorin administration adapted to methotrexate serum levels are required.

The efficacy of **cisplatin** against osteosarcomas was proven in early phase II trials. It has been subsequently incorporated into most multiagent regimens. Cisplatin therapy requires supportive hyperhydration. Oto- and nephrotoxicity are dose limiting toxicities.

Following positive phase II trials, **ifosfamide** has been part of many osteosarcoma protocols since the mid 1980s. Its efficacy may be related to the dose administered. Supportive measures necessary to prevent the otherwise frequent hemorrhagic uropathy after ifosfamide include hydration and the administration of Mesna (Uromitexan). Ifosfamide may also lead to chronic renal tubular toxicity, renal Fanconi syndrome, and sterility.

No **other agents** have come even close to replacing the four standard substances described above.

### **“Neoadjuvant” chemotherapy**

Currently, most institutions will use an approach consisting of preoperative chemotherapy (also called neoadjuvant chemotherapy or induction chemotherapy), followed by definitive surgery and postoperative, adjuvant chemotherapy. This approach facilitates subsequent limb salvage procedures, and also permits histologic evaluation of the resected specimen, to assess the effectiveness of therapy. Most investigators define good response as less than 10% viable tumor.

### **Prognostic factors**

Two treatment related variables are most important for outcome: incomplete surgery was the most important negative prognostic indicator in a series from the COSS study group, followed by a poor histological response to induction chemotherapy. It is currently unclear as to whether modification of therapy following definitive surgery in patients whose tumors demonstrate an inadequate response can affect outcome.

### **Local therapy**

Despite the efficacy of chemotherapy against microscopic disease, it cannot reliably control clinically detectable osteosarcoma. Therefore, surgery of the primary tumor and, if present, all metastases remains an integral part of successful therapy. Radiotherapy does not reliably sterilize osteosarcomas and is reserved for inoperable sites or those that can only be operated with inadequate margins. Osteosarcoma surgery has three aims: i) the tumor must be removed completely; ii) the patient should be left with good extremity function; iii) if possible, the appearance should be cosmetically acceptable. Complete tumor removal is of paramount importance. Until approximately 30 years ago, amputation was the only form of bone sarcoma surgery. This has since changed dramatically, and limb-salvage techniques are now used for the majority of cases. Even today, however, amputation may be the most appropriate type of surgery for selected patients with unfavorable tumor characteristics.

### **Treatment of primary metastatic and relapsed disease**

Detectable metastases must be removed by surgery if therapy is to be curative. As most

metastases develop in the lung, this usually implies thoracotomy. There is evidence that a significant proportion of patients with apparently unilateral lung metastases may, in fact, have bilateral disease. In general, acceptable surgical alternatives include bilateral thoracotomy or median sternotomy. Complete resection of osteosarcoma pulmonary metastases requires palpation of the lung, which is not possible thoracoscopically.

Approximately one quarter of all patients with proven metastatic disease at diagnosis and 40% of those who achieve a complete surgical remission of both the primary and all metastases in the context of an intensive polychemotherapy regimen will go on to become long term survivors. Patients with solitary primary metastases may have a prognosis similar to those with localized disease.

Osteosarcoma recurrences also usually involve the lung. Bone metastases and local recurrences are much less common, while other sites are rarely affected. Unfortunately, the survival rates after relapse are low, with less than 20% of affected patients becoming long term survivors. A short latency period and more than one or two metastases at relapse are associated with an especially poor outcome. Complete surgical removal of all sites of recurrence is the only therapeutic measure with unequivocally proven impact on survival. It may be prudent to irradiate suitable inoperable lesions in order to slow the progression of disease, but it is unlikely that this will lead to cure. The exact role of adjuvant chemotherapy in the treatment of operable relapsed osteosarcoma is still being debated. So far, success has been limited at best, and there is no accepted standard regimen outside of clinical trials.

### **Follow-up**

#### **Remission status**

Any follow-up for osteosarcoma must include regular assessments of the remission status as well as tests for possible late effects of (successful) treatment. Tumor directed follow-up should focus on the few organ systems where relapses are likely to occur. Pulmonary metastases, which are part of over 80% of recurrences, are potentially curable only as long

as they are resectable. They will most often not cause symptoms until they have reached a very large size or penetrate the pleura. In order to detect them at an earlier stage, lung metastases must be searched for by appropriate imaging studies, which include serial X-rays and/or CT-scans of the thorax. Local recurrences and bone metastases occur in a much lower percentage of patients and are often first detected because they cause symptoms, most noticeably pain.

### **Late effects**

Fortunately, many former osteosarcoma patients will go on to lead relatively normal and productive lives. Late complications may be caused by the tumor itself or by the surgery and chemotherapy needed to control it. Functional and cosmetic consequences for the musculoskeletal system depend on the location and extent of the osteosarcoma as well as the type of surgery employed.

The complications to screen for include, most prominently, cardiomyopathy (doxorubicin), renal dysfunction, either glomerular (cisplatin) or tubular (ifosfamide), and hearing loss. If end of treatment evaluations do not reveal impaired kidney function or hearing loss, it is unlikely to manifest later. High frequency loss is frequent, but only a minority of patients will require hearing aids. Protocols incorporating ifosfamide lead to sterility in most males and in some females. Secondary malignancies will develop in approximately 3% of patients within the first 10 years after treatment.

### **Perspectives**

Osteosarcoma remains a disease that presents challenges to the treating team. It is sensitive to a small number of medications, all of which have significant short and long-term toxicities. Radiation is of limited effectiveness. Even sensitive tumors are incompletely eliminated by chemotherapy. Surgical resection and reconstruction are therefore necessary, with their attendant morbidity and imposed requirement

for intensive rehabilitation. Patients with localized disease at initial diagnosis are cured approximately two-thirds of the time, whereas only one-third of those with initially metastatic disease achieve cure. Patients whose disease has recurred are difficult to cure, with a somewhat better outlook for those with isolated, resectable metastases.

New initiatives are required. These include the investigation of newer cytotoxic agents, singly or in combination. An example is the combination of trimetrexate and high dose methotrexate, designed to circumvent dysfunction of the reduced folate carrier present in many osteosarcoma cells at the time of initial diagnosis. A phase I study to first demonstrate tolerability of the combination is underway at Memorial Sloan-Kettering Cancer Institute. Other avenues must be sought as well. An example is the use of trastuzumab in patients who have, at initial diagnosis, widely metastatic tumors that express her2, as is currently being studied in the Children's Oncology Group. Another is the use of interferon alpha as maintenance therapy after the completion of standard cytotoxic chemotherapy, since it may have both anti-angiogenic and anti-neoplastic activity, as is planned in the upcoming European-North American (Euramos) study. Augmentation of the fas, fas ligand death pathway through the use of granulocyte-monocyte colony stimulating factor (GM-CSF) as an inhalation in patients with isolated pulmonary metastatic disease is being studied in the Children's Oncology Group. Investigations of the underlying oncogenic defect, possibly a loss of genomic control, are ongoing. In addition, increasing collaborative efforts with veterinary colleagues, to take advantage of the higher rate of osteosarcoma in large dog species are underway.

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# Acute Myeloid Leukemia

B. Lange & Brenda Gibson

## Introduction

In the past decade cooperative groups in France, Germany, Scandinavia, the United Kingdom, and the United States have reported 5-year survival rates of 50% or better in children and adolescents with acute myeloid leukemia (AML). Marked intensification of cytotoxic chemotherapy, judicious use of bone marrow transplantation, and standardization of supportive care have each contributed to improved outcome. Although refractory or recurrent AML remains the principal cause of death, treatment-related mortality in phase III AML studies is higher than in any other paediatric neoplasm, ranging from 6% to over 15%. It is now possible to identify variables related to host, disease, and treatment that predict success or failure. These predictions have led to the risk-stratification of therapy in contemporary trials. At the same time there is emerging a vast array of pharmaceuticals, biologics, and vaccines designed for targets that are relatively AML specific. These new agents offer hope for more effective, less toxic therapy in the future. Introducing them into paediatric trials is a priority.

## Treatment of AML

For many years paediatric AML therapy has relied on a chemotherapeutic backbone of cytarabine, anthracyclines and etoposide with or without marrow transplantation. Corticosteroids and thioguanine are included in some trials. The Medical Research Council (MRC) have developed a template based on a ten-day induction and post-remission therapy with 3 or 4 sequential combinations of high-dose cytarabine and alternating anthracyclines. The Children's Cancer Group (CCG) studies focus on intensive timing of 2 cycles of timed 5-drug induction and consolidation, followed by timed sequential high dose cytarabine or marrow transplantation. The Berlin-Frankfurt-Munster

(BFM) group uses an 8-day, 3-drug induction, high-dose cytarabine-based consolidation followed by a maintenance phase similar to paediatric ALL therapy and includes central nervous system prophylaxis often with cranial irradiation. Below we describe recent MRC and CCG studies and consider the role of marrow transplantation in first remission and the treatment of refractory and recurrent disease.

## MRC-AML10 and MRC-AML12 studies

The survival of children with AML in the UK treated on MRC trials has improved dramatically over the past 30 years (*Fig 1*). MRC trials conducted in the 1980s suggested that increasing the intensity of induction and consolidation therapy might lead to an improvement in outcome and identified the best induction regime as DAT 3+10 (daunorubicin, ara-C, thioguanine) followed by a modified second course of DAT 3+8; two courses of DAT 3+10 having been shown to be too toxic.

## MRC AML 10 (1988-1995)

1. Compared by randomization DAT 3+10 with ADE 3+10+5 (ara-C, daunorubicin, etoposide) as induction chemotherapy. These regimens delivered the same doses of daunorubicin and ara-C and therefore tested the comparative benefits of thioguanine and etoposide. There was no significant difference in CR rate (DAT 90% v ADE 93%), induction deaths (4%) or resistant disease (DAT 4% v ADE 3%). Overall survival, DFS, EFS were similar at 10 years (DAT 57% v ADE 51%,  $p=0.3$ ; DAT 53% v ADE 48%,  $p=0.3$ ; DAT 48% v ADE 45%,  $p=0.5$  respectively). There was no evidence that children with monocytic involvement (FAB type M4 or M5) did better with an etoposide containing regimen, although this had previously been reported.

2. Investigated the role of BMT following 4 courses of intensive chemotherapy - DAT 3+10 or ADE 3+10+5, DAT 3+8 or ADE 3+8+5, MACE (amsacrine, ara-C, etoposide), MiDAC (mitoxantrone, high dose ara-C).

a) Children with a sibling donor were recommended for an allo-BMT in 1<sup>st</sup> CR; a genetic randomization. Analysed on donor availability ie intention to treat, allo-BMT was associated with a significant reduction in relapse risk from 45% to 30% at 10 years ( $p=0.02$ ), but this did not translate into a survival advantage (donor 68% v no donor 59%,  $p=0.3$ ). The fewer relapses in the donor group were counter balanced by procedure related deaths (11%,  $p=0.001$ ) and patients who relapsed after allo-BMT were not salvaged, whilst a percentage of those who had received chemotherapy alone were.

b) Children who did not have a donor were randomized to an A-BMT or no further treatment. Again at 10 years, there was no significant overall survival advantage for A-BMT (A-BMT 70% v NFT 58%,  $p=0.2$ ), but there was a decrease in the relapse risk (A-BMT 31% v NFT 52%,  $P=0.03$ ) and a significant improvement in DFS (A-BMT 68% v NFT 44%,  $p=0.02$ ). The reduction in the relapse risk did not translate into a significant improvement in overall survival because children who relapsed and who had not had an A-BMT were more likely to be salvaged with second line treatment than those who had had an A-BMT.

Therefore MRC AML 10 failed to show a survival advantage for either allo-BMT or A-BMT in 1<sup>st</sup> CR, although both were associated with a reduction in the relapse risk. The failure to translate this into an improvement in overall survival differed by type of transplant.

3. AML 10 allowed stratification of patients into good, standard and poor risk groups based on their cytogenetics and response to the first course of treatment. Good risk patients are those with favourable genetic abnormalities - t(8,21), t(15,17), inv(16) irrespective of their

bone marrow status after course 1 of therapy. Standard risk patients are those with neither favourable or adverse cytogenetic abnormalities and not more than 15% blasts in their bone marrow after course 1 of therapy. Poor risk patients are those with more than 15% blasts in their bone marrow after course 1 of therapy and adverse genetic abnormalities - -5, -7, del(5q-), abn (3q), complex karyotype (>/- 5 abnormalities).

Survival from 1<sup>st</sup> CR for these risk groups was 77%, 58% and 30% respectively and relapse rates 35%, 43% and 72% (both  $p<0.0001$ ) at 10 years.

### MRC AML 12 (1995-2002)

This trial took forward the marginally better induction regimen from MRC AML 10 (ADE-when paediatric and adult results were combined) and the standard MRC template became ADE, ADE, MACE, MiDAC.

1. MAE (mitoxantrone, ara-C, etoposide) was compared to ADE; mitoxantrone being potentially less cardiotoxic than daunorubicin. The CR rates are similar (ADE 92% v MAE 90%) and the estimated probability for 5-year survival is not significantly different (ADE 64% v MAE 70%,  $p=0.1$ ).

2. The reduction in relapse risk, similar for A-BMT and allo-BMT, in AML 10 suggested that the benefit might be one of an additional block of treatment rather than a graft versus leukaemia effect. The benefit of additional treatment was tested by the introduction of a fifth course of therapy in a randomized fashion. HD ara-C with asparaginase was chosen to avoid further anthracycline exposure. To date there is no benefit for an additional block of treatment suggesting that the ceiling of benefit for chemotherapy may have been reached with four blocks of treatment although the results of this trial are immature and follow-up continues.

3. Treatment was stratified by risk group. The risk group stratification derived from MRC AML 10 remains prognostically significant in MRC AML 12.

4. Good risk patients were not eligible for BMT in first CR, but standard and poor risk patients were. Although no benefit in OS had been

demonstrated for allo-BMT in AML 10, other co-operative groups reported benefit, and it seemed reasonable to continue to investigate the role of allo-BMT. A-BMT was not employed in AML 12 in the absence of a demonstrable benefit in OS and because of its potential long-term morbidity. When results from MRC AML 10 and 12 are combined, they show a significant reduction in relapse risk ( $2p = 0.02$ ), which does not translate into a significant reduction in DFS ( $2p = 0.06$ ) or OS ( $2p = 0.1$ )

5. At present the results of MRC AML 12 are superior to MRC AML 10. The estimated probability of 5 year OS, EFS, DFS are 66%, 56% and 61 % respectively.

### **CCG-2891, CCG-1941, and CCG 2961 studies**

In the 1980s and early 1990s conventional induction therapy involved 7 days of chemotherapy, a week of rest, and examination of the marrow on day 15 to assess efficacy: if there was residual leukemia, therapy was repeated; if there was <5 percent blasts, therapy was delayed until recovery of trilineage hematopoiesis. CCG-2891 (1989-1994) tested 2 hypotheses: 1) compared to conventional induction, an intensively-timed induction would more effectively reduce the leukemic population; and 2) that matched related donor bone marrow transplantation (MRD BMT) would achieve higher disease-free survival and overall survival than either purged autologous marrow transplantation (ABMT) or high-dose ara-C based chemotherapy. Intensive timing induction consisted of dexamethasone, cytarabine, thioguanine, etoposide and daunomycin (rubidomycin) (DCTER) given over 4 days, rest for 6 days, and repetition of the same drugs on day 11 regardless of marrow status and patient condition. A second course of the same therapy was repeated in phase 2. In Phase 3 patients with matched related donors underwent BMT and those without donors were randomly assigned to ABMT or chemotherapy. Intensive timing consisted of dexamethasone, cytarabine, thioguanine, etoposide and daunomycin (rubidomycin) (DCTER) given over 4 days, rest for 6 days, and repetition of the same drugs on days 11 through 14. regardless of marrow status and patient condition.

Excluding patients with Down syndrome, acute promyelocytic leukemia, myelodysplastic syndrome and secondary AML, 318 patients were randomized to conventional timing and 556 received intensive timing, 299 of whom received granulocyte-colony –stimulating factor starting on day 6. Treatment-related mortality in 2 courses of induction was 4% with standard timing, 13% with intensive timing ( $p = 0.05$ ) and 8% with intensive timing plus G-CSF; respective induction failure was 23%, 10% and 10%. Despite a significantly higher induction mortality, intensive timing effected a significantly higher EFS (44+4% vs. 27 +5%), DFS (60+4% vs. 37+5%) and OS (49+4% vs. 34+5%) at 9 years from on study.

In CCG-2891 MRD BMT was significantly better than either ABMT or chemotherapy. Patients DFS and OS of patients who received intensive timing induction was consistently superior to that of standard timing in all 3 post-remission regimens implying that better induction breeds post-induction results. EFS and OS with and without G-CSF were nearly identical.

CCG-2891 demonstrated that despite a significantly higher induction mortality, intensive timing effected a significantly higher EFS and OS. In this study MRD was significantly better than either ABMT or chemotherapy. Patients DFS and OS of patients who received intensive timing induction was consistently superior to that of standard timing in all 3 post-remission regimens implying that better induction gives rise to better post- induction results.

CCG-2941 (1994-1996) was a pilot study to test feasibility of replacing 4 mg of daunorubicin with 1 mg idarubicin (IdaDCTER) and eliminating 3 cycles of lower dose chemotherapy used in CCG-2891. After enrollment of 60 patients, interim analysis showed a 12 percent toxic mortality and 20 percent withdrawal. The replacement of daunorubicin with idarubicin on days 1-4 and 11-14 was deemed unfeasible in the intensive timing paradigm. The second idarubicin was replaced by daunorubicin and achieved a toxicity profile similar to that of daunomycin alone in CCG-2891.

CCG-2961 (1996-2002) used the Idarubicin/ daunorubicin induction in all patients. It tested the hypothesis that changing the Phase II

consolidation therapy to a potentially non-cross-resistant combination, fludarabine, cytarabine, idarubicin, would achieve higher DFS and OS and that administering Interleukin-2 after high-dose cytarabine intensification would recapitulate the effects of graft-vs-leukemia of MRD BMT. The study was suspended in 1999 because of a projected treatment-related mortality in excess of 12%. It was reopened after 9 months with mandated standardized supportive care guidelines.

Among 900 patients with de novo AML, remission induction rate was 87%; OS, 53+3%, and EFS is 44+3%, at 3 years. The biggest difference in this study occurred in the comparisons of the 495 patients enrolled prior to suspension and the 404 enrolled after suspension: EFS 41+4% vs. 47+5% OS 52+5% and 63+5% ( $p=0.003$ ) pre and post-suspension respectively. ( $P=$ ).

#### **Bone marrow and stem cell transplantation in first remission**

There is considerable debate concerning which patients should receive MRD BMT in 1st CR. While trials in the United States and France have demonstrated that compared to intensive chemotherapy or ABMT, MRD BMT significantly improves EFS and OS, the MRC AML-10 and the BFM 93 trial in Germany have not shown a statistically significant advantage to MRD BMT. The combined paediatric and adult experience in MRC AML10 demonstrated a significant benefit to ABMT compared to no further therapy after 4 courses of intensive chemotherapy, which emerged late at two years. These discordant results may arise from the differences in patient populations and treatment protocols of each cooperative group, the statistical limitations of relatively small numbers, and the biases of parents, guardians and physicians regarding post-induction therapies. As of 2005, a consensus is emerging that MRD BMT in 1<sup>st</sup> CR is unnecessary for paediatric patients with AML with the cytogenetic abnormalities  $t(15;17)$ ,  $t(8;21)$ , and  $(inv\ 16)$ . In most recent studies, transplant-related mortality for MRD in 1<sup>st</sup> CR is <10 per cent, but some survivors have graft vs. host disease, and almost all are infertile. Patients who receive chemotherapy only do not have these problems. Some paediatric groups favor busulfan and cyclophosphamide cytoreduction

rather than total body irradiation (TBI) plus an alkylating agent; however, it is not clear that cytoreduction with chemotherapy only is either less toxic or more or less efficacious than TBI.

#### **Refractory and Recurrent AML**

Refractory disease and relapse remain the major causes of treatment failure in AML. Patients with refractory disease or with relapse occurring within a year from diagnosis of AML have a poor prognosis: less than 20 percent of patients survive 2 years. High dose cytarabine with an anthracycline other than that used previously is the most common salvage regimen. In most cases once remission is achieved stem cell transplantation is considered the treatment of choice. Since many patients with matched related donors have already had a MRD BMT, the majority of patients received transplants from alternative donor sources. If there is no matched related donor, MUD transplant, or in younger, smaller patients, cord blood transplant may be an accessible alternative. Most cytoreduction in the setting of relapse includes TBI. Treatment-related mortality with alternative donors is 20-30%. Recent studies in adults are demonstrating some success with the non-myeloablative stem cell transplantation using low-dose (200cGy) TBI and cytoreduction that includes fludarabine monophosphate; whether this will be superior to myeloablative transplantation remains to be seen. Recently, new biologic therapies that may be more effective and less toxic have been introduced into AML therapy. Gemtuzumab ozogamicin, an anti-CD-33 antibody linked to calicheamicin, has sometimes induced complete remissions in recurrent adult and paediatric AML. Inhibitors of farnesyl transferase an enzyme involved in RAS activation have had activity in unfavorable AML in older adults and in juvenile myelomonocytic leukemia in infants. Vaccines targeting WT1, the Wilms tumor gene, telomerase, surviving, and homeobox genes have shown efficacy in preclinical trials. Many of these agents are expected to be most useful in early remission, in the setting of minimal residual disease.

Most relapses are marrow relapses. There is no standard approach to management of extramedullary relapses. There are reports of occasional control with local treatment only. However, in most cases extramedullary relapse

is a harbinger of marrow relapse. The role of local irradiation, new systemic therapy, and stem cell transplantation remain to be defined.

## Prognostic Factors And Risk-Stratification

### 1. The Host

Table 1 lists factors related to host, disease and treatment that are predictive of outcome. Among the host-related factors, genetic predisposition to AML probably has the most profound impact on outcome. There is general agreement the patients with Down Syndrome (DS) or Down Syndrome mosaicism under 5 years of age have a significantly better outcome than other patients with AML. In DS, AML typically takes the form of a megakaryoblastic leukemia that is especially sensitive to cytarabine. Patients with DS are often treated according to separate protocols that are substantially less intensive than contemporary AML phase III trials. In contrast, patients with germline marrow failure syndromes such as Fanconi anemia, Kostmann syndrome, and Shwachmann-Bodian-Diamond syndrome are excluded from the phase III trials because they cannot tolerate therapy with anthracyclines and alkylating agents, and they do not have the ability to restore trilineage hematopoiesis following therapy. Transplantation with reduced-intensity cytoreduction before occurrence of AML is the treatment of choice.

Age is also an important prognostic factor with younger being better. About 10 percent of neonates with Down syndrome have a transient megakaryoblastic leukemic disorder that in most cases regresses spontaneously; in up to a third it recurs in later infancy as the favorable form of megakaryoblastic leukemia described above. Occasionally AML in genotypically normal neonates regresses spontaneously. When DS infants are excluded in most studies outcomes of the remaining infants are similar to those of children over age 2 years.

As a group children fare better than adolescents, and in the MRC studies, children and adolescents had significantly better outcomes than adults under age 45 years. In most studies there is no significant difference in outcomes of males and females. In the CCG studies black patients experience a significantly inferior EFS and OS compared to white patients; ethnicity has

not been addressed in most other studies. Also in the CCG studies, presence of a matched family donor is associated with better outcomes.

### 2. The Disease

Factors related to disease are white blood cell count at diagnosis, extramedullary disease, FAB morphology, immunophenotype, cytogenetics and most recently a few molecular markers. A white blood cell count under 20,000/mm<sup>3</sup> is generally favorable and over 100,000 is unfavorable. Isolated chloromas were favorable in CCG-2891; patients without clinically detectable marrow disease require systemic therapy. The impact of CNS disease at diagnosis is variable.

The impact of FAB subtype varies from study to study. Megakaryoblastic leukemia, FAB M7 or erythromegakaryoblastic leukemia (FAB M6/M7) in patients who do not have Down syndrome generally has a poorer outcome than the other FAB types. Early BFM studies noted that eosinophilia was favorable, but this may reflect overlap between the FAB M4 eo phenotype and the inversion 16 genotype.

Blasts from over 90 percent of paediatric patients with AML express one or more myeloid-associated surface antigens CD13, CD14, or CD33; 30 percent of cases show expression of B-lymphocyte antigens, 60 percent express T-lymphocyte antigens. Expression of lymphoid-related antigens is of no prognostic significance. There is no consensus about favorable or unfavorable immunophenotype. Expression of combinations of antigens is highly patient specific and can be used to monitor minimal residual disease.

In over 70 percent of cases AML, the blasts have an abnormal karyotype. There is some overlap between immunophenotype, morphology, and cytogenetic findings. CD19<sup>+</sup> and CD56<sup>+</sup> are associated with FAB AML M2/t(8;21); CD2<sup>+</sup> and CD7<sup>+</sup> with FAB AML M2/t(8;21 negative); and CD33<sup>+</sup>, CD34<sup>-</sup> with FAB AML M3/t(15;17). In these cases it is the cytogenetic marker that determines outcomes. There is now general agree t(8;21), inv(16), and t(15;17) are relatively favorable subtypes. Patients with t(15;17) have acute promyelocytic leukemia (APL). In many cooperative groups, patients with APL are treated with combinations that emphasize all-trans

retinoic acid and anthracyclines. APL is also particularly sensitive to gemtuzumab, ozogamicin and arsenic trioxide. In both paediatric and adult studies, monosomy 7 or 7q- genotype is associated with poor outcome. Retrospective studies show that allogeneic stem cell transplantation is the treatment of choice, but there are no studies that allocate these patients to alternative donor transplants in first remission.

There are many studies in AML in adults and several in paediatrics that show that internal tandem duplication of the receptor for FLT3 ligand (FLT3 ITD) is associated with a poor outcome. This is the only molecular marker for which the results are consistent across all studies. Overexpression of P-glycoprotein has been associated with multiple drug resistance in vitro and in vivo. However, results of clinical trials are mixed, and inhibiting P-glycoprotein has not resulted in significant improvements in AML.

### 3. The Treatment

Treatment is obviously an important variable; with the exception of AML in some neonates, without treatment AML is fatal and randomized studies indicate that some treatments are better than others. How rapidly the treatment reduces the burden of leukemia is an important predictor of outcome. Standard measures of response are the blast percentage on the day 15 marrow and achievement of remission after one or two courses of therapy. A day 15 marrow with less than 5 percent or less than 15% blasts portends a higher probability of survival. Failure to reduce the blast percentage to <5% and regenerate trilineage hematopoiesis after the first course of therapy predicts a relatively high probability of treatment failure. However, the majority of patients who experience relapse are in neither of these groups; hence more sensitive indicators of residual disease are needed. Multichannel flow cytometric assessment of marrow to detect 0.1% residual blasts at the end of induction identifies a larger population of patients at risk for relapse. PCR-based analyses are suitable for detection of 0.01% to 0.0001% residual blasts. However, rare blasts with t(8;21) are present in the majority of patients who are in remission and remain in remission for years. In this case an increase in the numbers of PCR-detected cells may predict future relapse.

Another parameter of treatment is supportive care. In both the MRC 10 and CCG-2961 studies, implementation of standardized supportive care guidelines reduced the treatment-related mortality. It is likely that experience with a protocol is important as well. In CCG-2961 the mortality was reduced and EFS and OS improved after the first 18 months before the institution of mandatory guidelines. The improvement could not be accounted for solely by reduction in mortality but may include nuances in care that reduce morbidity as well.

### Conclusion

The outlook for children with AML continues to improve. Similar and encouraging results have been obtained by a number of co-operative groups provided that intensive regimens with anthracyclines and HD ara-C are used. MRC results suggest that there may be a ceiling of benefit from chemotherapy and that further improvement in leukaemia control will be dependent on alternative approaches. The benefit of A-BMT is probably at best that of additional treatment. The role of allo-BMT in 1<sup>st</sup> CR continues to be tested. However, as the results from intensive chemotherapy continue to improve, the potential benefit of allo-BMT may become more limited and restricted to certain groups of patients, particularly alternative donor transplant. Monoclonal antibodies, drugs targeted at specific fusion genes and multi-drug resistance modifiers are being tested. Trials combining Gemtuzumab with intensive chemotherapy are ongoing. Toxicity can be limited by restricting transplantation and reducing anthracycline exposure where appropriate.

Risk group stratification will allow the targeting of therapy to risk. Cytogenetics and the speed of response to treatment have already been identified as prognostically significant. MRD monitoring, gene expression profiling and the identification of additional prognostic indicators eg FLT 3 may further define risk. The low CNS relapse rate in AML with intrathecal CNS directed treatment alone challenges the use of cranial irradiation with its long-term sequelae. Finally, AML is a heterogeneous disease with a number of rare subtypes, the treatment of which can only be addressed by international collaboration.

**Table : Factors Predictive of Outcome in Paediatric AML**

	Favorable	Unfavorable
<b>The Host</b>		
<i>Genetic</i>		
Down Syndrome	••••	
Fanconi anemia		•••
Shwachmann-Diamond		•••
Kostmann Syndrome		•••
Matched related donor	••	
<i>Age</i>		
Infant	••	
Child	••	
Adolescent		••
<i>Ethnicity</i>		
White	••	
Black		••
Hispanic		••
<i>Body Mass Index</i>		
Normal (10-95 %)		
Underweight (<10 %)		••
Obese (> 95 %)		••
<b>Disease</b>		
<i>White Blood Cell Count</i>		
<20,000/mm <sup>3</sup>		••••
>200,000/mm <sup>3</sup>		••••
<i>Morphology</i>		
M7, M/67		••
<i>Cytogenetics</i>		
del (7), 7q-		••••
t(8;21)	•••	
t(15;17)	••••	
inv(16)	••••	
complex karyotype		••••
<i>Molecular genetics</i>		
FLT3 ITD		••••
P-glycoprotein expression	•	
<b>Treatment</b>		
Early Response	••••	
Induction Failure		••••

Legend: Each • corresponds roughly to the level of evidence and the strength of agreement in the paediatric oncology community.

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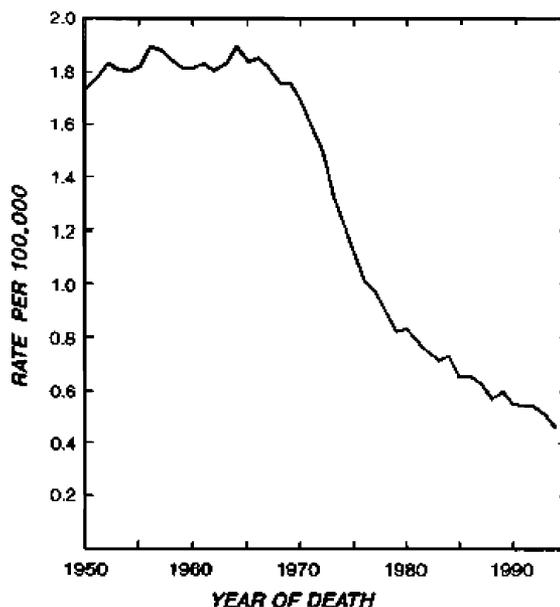
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# Hodgkin's Lymphoma –Current Concepts

Sachdeva A, Wallace WHB

Inspection of the Hodgkin's Lymphoma (HL) mortality curve (Fig 1) shows a dramatic improvement in survival over the past 30 years. In the United States, mortality remained above 1.8 per 100,000 per year in the 1950s and early 1960s, but decreased to 0.47 by 1994.<sup>1</sup> Whereas HL accounted for 30% of total lymphoma deaths in 1950, it accounted for only 6% (1,440 US deaths) in 1994.



**Figure 1** : HD mortality in white males and females in the United States from 1950-1994. (Reprinted from Ries et al.<sup>1</sup>)

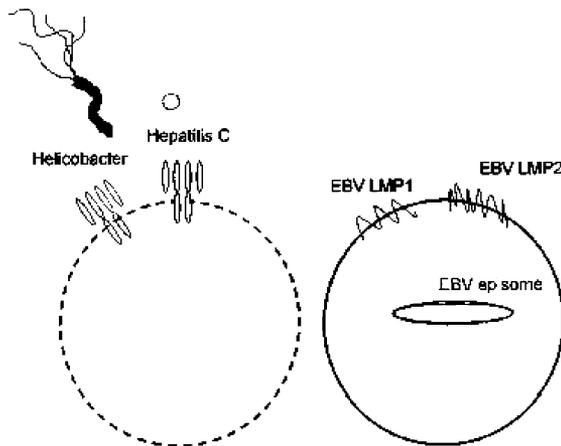
Juan A. del Regato, 1909-1999, was a superb clinician-educator who recognized the radio curability of Hodgkin's Lymphoma but questioned treatment without late effects, particularly in children. The remarkable progress in paediatric Hodgkin's disease today is a tribute to this influential pioneer, who served as a role model to many. Combined modality therapy using low-dose, involved-field radiation and multiagent chemotherapy today results in a 5-

year relative survival rate of 94% among American children with Hodgkin's disease.<sup>2</sup> However, several areas hold promise for future advances, new noninvasive staging techniques, including 18F-fluorodeoxyglucose-positron emission tomography; the definition of risk groups on the basis of a prognostic index, facilitating risk-adapted therapy. Finally, novel therapies, such as the anti-CD20 antibody, rituximab, may be useful for children with CD20+, lymphocyte-predominant Hodgkin's disease. The universal goal of cure without late effects is realistic for almost all children with Hodgkin's disease today.

## Epidemiology Of Hodgkin's Lymphoma And Epstein-Barr Virus

Hodgkin's "disease" is a B cell lymphoma.<sup>3</sup> The mystery embodied in the term Hodgkin's disease, which defines neither the affected tissue nor the disease process, is underscored by the name of the cell required for diagnosis: Reed-Sternberg. The malignant character of the disease is now beyond dispute and the cellular lineage is clearly understood to be B cell. The explanation for the difficulty in identifying the tumor may be attributed to two of its defining characteristics: the tumor cells are rare in the mass of the tumor and fail to express many B cell markers—most notably immunoglobulin.

Whereas the incidence of non-Hodgkin's lymphoma has been rising since World War II, the incidence of Hodgkin's lymphoma has been flat. Two age-incidence peaks have long been recognized in North America and Western Europe: young adult and older adult. In developing countries and in parts of Asia, the young adult peak is much less prominent or even absent. Several epidemiologic studies suggested an association with small family size and other factors that might result in delayed exposure to common viral infections



**Figure 2 :** Different pathways lead to lymphoma.

### Epstein-Barr Virus and Hodgkin's

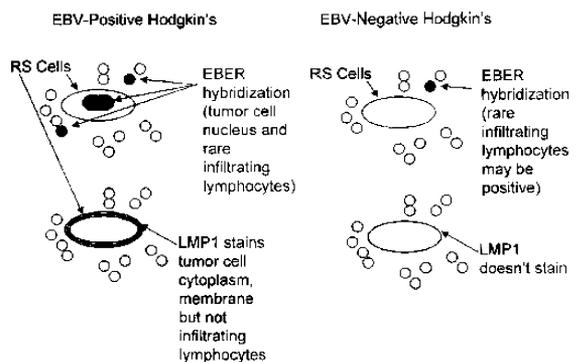
Serologic studies have suggested modestly higher titers to Epstein-Barr virus (EBV) antigens in patients with Hodgkin's lymphoma than in controls, but the differences in titers have been modest. EBV is a ubiquitous virus and the vast majority of Hodgkin's patients (and adults) are EBV seropositive.

### Infectious Agents and Lymphomagenesis

There are two very different pathways by which infectious agents drive malignant lymphoproliferative diseases: some infectious agents activate extrinsic pathways through lymphocyte antigen receptors, while others activate intrinsic pathways bypassing antigen receptors<sup>4</sup> (**Figure 1**). *Helicobacter* drives the proliferation of B cells in an antigen-specific manner and treatment with anti-infective agents sometimes results in tumor regression. Hepatitis C may similarly act through immunoglobulin receptors and may similarly respond to treatment with anti-infective agents.<sup>5</sup> In contrast, human T cell leukemia virus-1 (HTLV1) drives the proliferation of infected T lymphocytes independent of the specificity of the T cell receptor.<sup>6</sup> Similarly, in post transplant lymphoproliferative disease, EBV drives lymphoproliferation independent of the specificity of the immunoglobulin. Several different EBV genes are required to act in concert to bring about this transformation, including five of the six EBV nuclear antigens.

### Detection of EBV in Tumor

EBV nucleic acids and proteins have been identified in the tumor cells in a subset of patients. Approximately 30% of Hodgkin's lymphoma in the United States and North America harbor these nucleic acids and proteins, while much higher percentages of tumor cells show evidence of virus in some developing countries (approaching 100% in parts of Latin America, Africa and Asia).<sup>7</sup> Formal guidelines for interpretation and comparisons of methods for detecting virus in fixed tissues have recently been published<sup>8,9</sup> (**Figure 2**). With some well-documented exceptions EBV is present in each of the tumor cells in a particular patient at all sites of disease, at presentation and at relapse.



**Figure 3 :** Detection of Epstein-Barr virus (EBV) in clinical specimens.

Those regions of the world in which Hodgkin's lymphoma is most consistently associated with EBV are those regions in which the syndrome of infectious mononucleosis is least common. On the other hand, regions in which infectious mononucleosis is most common have been those regions where the presence of EBV in tumor is least common. Evidence has very recently been presented that EBV upregulates expression of activation-induced deaminase, the enzyme responsible for somatic hypermutation in germinal center B cells.<sup>10</sup> EBV need not leave any viral genetic sequences behind as a footprint. In contrast with tumor viruses whose DNA integrates into host cell DNA, loss of EBV episomes from malignancies in tissue culture is well documented in EBV tumor cell lines.<sup>11</sup> In a population-based analysis of acute infectious mononucleosis and malignancy in Denmark and Sweden<sup>6</sup> the relative risk of EBV-positive Hodgkin's lymphoma was increased fourfold in

patients with serologically confirmed infectious mononucleosis. There was no increase in the relative risk of EBV-negative Hodgkin's lymphoma. The median estimated time interval from the diagnosis of mononucleosis to EBV-positive Hodgkin's lymphoma was approximately 4 years. Thus infectious mononucleosis is clearly linked to EBV-positive Hodgkin's lymphoma and there is no evidence to suggest that it is linked to EBV-negative Hodgkin's lymphoma.

### Other Infectious Agents and Hodgkin's Lymphoma

The incidence of Hodgkin's lymphoma is increased in incidence among human immunodeficiency virus (HIV)-infected patients.<sup>12</sup> In contrast to non-Hodgkin's lymphomas that are "AIDS-defining" malignancies, the increased incidence of Hodgkin's lymphoma is more subtle. The increased risk of Burkitt's lymphoma and brain lymphoma in AIDS patients is hundreds- or thousands- fold, whereas the increased incidence of Hodgkin's lymphoma in AIDS is approximately 3- to 10-fold. A recent population-based study of HIV Hodgkin's lymphoma in the San Francisco Bay area showed that 90% of Hodgkin's tumors in HIV patients were EBV-positive, that nodular sclerosis histology was only half as common among HIV-infected patients as in the general population, and that lymphocyte-depleted disease was much more common.<sup>12</sup> HIV patients were also more likely to have advanced-stage disease. Whether the contribution of HIV to Hodgkin's lymphomagenesis is simply related to generic immunodeficiency or reflects some more specific contribution of particular HIV proteins or HIV-induced immune dysregulation is not clear. However, it is worth bearing in mind that the CD4 T cell count in HIV Hodgkin's is typically much higher than that in some other EBV-associated malignancies in HIV patients, such as brain lymphoma. Interesting evidence regarding measles has also been presented.<sup>13</sup> Immunohistochemical, reverse transcriptase-polymerase chain reaction and in situ hybridization studies have shown evidence of measles virus in Hodgkin's tumor tissues in slightly more than half of patients. These include patients with and without EBV in their tumors.

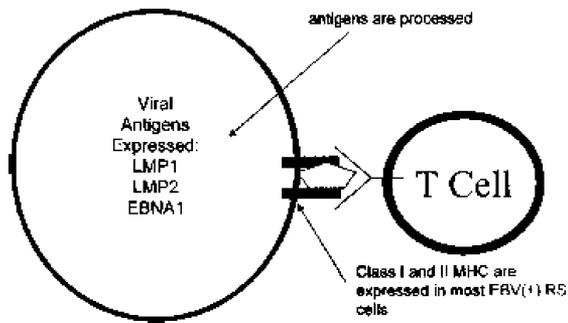
Any contribution to pathogenesis remains highly speculative at this point. Despite extensive investigation, no evidence has emerged to support a role for cytomegalovirus, varicella-zoster virus, mumps, pertussis, human herpesvirus 6, 7, or 8, JC virus, adenovirus or HTLV1 or 2.

### Viral Proteins in Hodgkin's Pathogenesis

The EBV latency membrane protein 1 (LMP1) has been recognized as a likely contributor to tumorigenesis. Expression of the protein is transforming in immortalized murine cell lines and leads to lymphoma when expressed in B cells in a transgenic model.<sup>14</sup> The protein is a member of the tumor necrosis factor receptor (TNFR) superfamily and most closely resembles CD40. However, in contrast to CD40, LMP1 signaling is constitutively active and requires no ligand. Among the multitude of activities of LMP1, it activates NF- $\kappa$ B by promoting the turnover of I $\kappa$ B  $\alpha$ . Latency membrane protein 2A (LMP2A) also seems likely to play an important role. As noted above the tumor cells of Hodgkin's lymphoma do not express immunoglobulin genes. B cells that lack functional Ig would be expected to die by apoptotic mechanisms as has clearly been shown in transgenic mouse models. However, LMP2A has been shown to provide a tonic signal that mimics that associated with immunoglobulin expression so as to prevent apoptosis of such cells—at least in a murine model.<sup>15</sup> The role of this viral protein in preventing apoptosis in tumor cells suggests the possibility that related pathways may be targeted by small molecules for therapeutic purposes.<sup>16</sup>

### Does EBV in Hodgkin's Lymphoma Provide a Suitable Target for Immunotherapy?

Viral antigens expressed in Hodgkin's lymphoma are commonly recognized by CD8 T cells (**Figure 3**).<sup>17,18</sup> The antigens are generally not mutated in Hodgkin's lymphoma.<sup>19</sup> Most EBV-associated Hodgkin's tumors express class I MHC antigens and studies of cell lines suggest that the antigen-processing machinery is intact. Studies using adoptive cellular immunotherapy approaches have been initiated with limited success to date.<sup>20</sup> However, improved targeting of the T cell lines to antigens such as LMP2A actually expressed in Hodgkin's lymphoma holds promise as may therapeutic vaccine strategies



**Figure 4** : Epstein-Barr virus (EBV) Hodgkin's as an immune target.

### The Utility Of Pet In Managing Patients With Hodgkin's Lymphoma

Fluorodeoxyglucose (FDG)–positron emission tomography (PET) has a number of potential advantages for the hematologist-oncologist in refining and improving the management of Hodgkin's lymphoma. (see figure 4)

#### Staging

Hodgkin's lymphoma is generally treated according to stage and risk profile; therefore, staging provides the basis for different treatment strategies such as RT, chemotherapy or a combination of both. Recently, metabolic imaging with PET has been increasingly used in the management of lymphoma patients. Since PET relies on the detection of metabolic alterations observed in cancer cells, this examination yields data that are independent of associated structural characteristics. Furthermore, the ability to perform whole body imaging within one examination without increasing the radiation burden makes PET an ideal technique to "screen" patients for cancer deposits. The most frequently used tracer is the glucose analogue FDG. The use of FDG for in vivo cancer imaging is based upon the higher rate of glucose metabolism of cancer cells compared with nonmalignant tissue. With the exception of small lymphocytic and MALT lymphomas, most lymphomas, including Hodgkin's lymphoma, exhibit moderate to high FDG uptake.<sup>21</sup> Several studies have investigated the role of FDGPET for the initial staging of lymphoma (see also a recent review of Schiepers et al<sup>22</sup>).

Compared with gallium scintigraphy, FDG-PET appears to have advantages that include inherent superior resolution, higher sensitivity (especially in the abdomen and bone), lower radiation burden (10 mSv/PET versus 44 mSv/gallium scintigraphy) and a shorter examination time (2 hours for PET versus 3 days for gallium scintigraphy).<sup>23,24</sup> FDG-PET was also found to be more sensitive and specific than bone scintigraphy for the detection of cortical bone involvement<sup>25</sup> and complementary to bone marrow biopsy for detection of marrow involvement distant from the biopsy site.<sup>26</sup> Most reports have focused on comparisons of PET with CT.<sup>22</sup> In most cases, PET was found to be more sensitive for detecting of both nodal (e.g., small sized nodes) and extra-nodal (especially spleen and bone) involvement, but PET-negative, CT-positive lesions do occur in a small number of cases. High FDG uptake in brown fat tissue or muscle can hamper the interpretation of the head and neck and mediastinal regions. Moreover, physiological uptake in gut and the renal collecting system can obscure evaluation of lymphoma in adjacent nodal sites. Therefore, optimum use of FDGPET is likely in combination with CT. Combined use of PET-CT can improve accuracy by increasing the certainty of diagnosis in those difficult regions.<sup>27</sup> Incorporating PET in the initial staging in lymphomas results in upstaging or downstaging in 10%– 20% of patients, but the impact on treatment management is less obvious. Naumann et al<sup>28</sup> analyzed the potential impact of PET staging on therapy decision in 88 patients with Hodgkin's lymphoma. Concordant findings between PET and CT were found in 70/88 patients (80%). In 11 patients (13%), PET detected additional sites, which would have resulted in treatment intensification in 9 of them, all with early stage disease. Focusing on the patients with stage IA-IIB disease only (n = 44), treatment would have been intensified in 20%. Compared to conventional diagnostics, PET downstaged 7 patients (8%) but this was only correct in 1 patient (inflammatory enlarged cervical node). False negative PET findings would have erroneously led to a minimization of therapy in 6 patients (7%). Therefore, FDGPET should not be used instead of but in combination with conventional diagnostics.

### Evaluation of Residual Masses After Treatment

Approximately two-thirds of patients with Hodgkin's lymphoma will have a residual mass seen with standard imaging tests at the end of treatment, but only 20% of these patients will eventually relapse. Several recent reports have shown the effectiveness of FDG-PET in the evaluation of residual disease at the end of treatment, including studies assessing patients with Hodgkin's lymphoma.<sup>29-32</sup> A high negative predictive value (NPV) of FDG-PET (81%–100%) has been consistently reported by most studies, clearly showing the ability of PET to identify patients with an excellent prognosis. The question of whether, after first-line chemotherapy, RT can be omitted in patients with a negative PET scan is still unanswered. Prospective randomized trials are needed to compare the PFS, overall survival (OS) and long-term complications observed in patients with a negative PET after chemotherapy who receive no further therapy or standard RT as planned at the initiation of treatment.

### Early Response Monitoring for Risk Stratification

Since treatments that are more aggressive, but also more toxic, are available, there is a growing interest in the use of risk-directed approaches to utilize prognostic factors that predict for relapse.

The duration of a complete remission (CR) and other long-term outcomes might be more affected by the sensitivity of the tumor to chemotherapy than by the prognostic factors seen at presentation. Promising results with FDG-PET can be obtained when evaluating treatment response. Treatment-induced changes resulting in tumor cell death or growth arrest reduce FDG uptake, making this a sensitive and early marker of response.<sup>33</sup> Romer et al<sup>34</sup> described a rapid decrease of FDG-uptake in patients with NHL as early as 7 days after commencing treatment. However, FDG-uptake at 42 days correlated better with long-term outcomes; early FDG reduction probably reflects initial chemosensitivity of the tumor whereas results of later evaluations are more related to the detection of resistant clones. Since that initial description, several studies have correlated PET

(PPV) of PET is more variable (25%–100%). When PET images are interpreted in correlation with clinical history and CT by radiologists/nuclear medicine physicians with specific expertise in PET, the PPV of PET in patients with Hodgkin's lymphoma is probably equivalent to that observed in NHL (> 80%) and residual positivity is highly suggestive of residual lymphoma for which additional treatment should be considered. In equivocal cases close follow-up or additional diagnostic procedures may be warranted to reduce risks of giving additional treatments that are based on false-positive results.

### Current Concepts In Management Of Hodgkin's Disease

Paediatric Hodgkin's disease is one of the most curable of childhood malignancies today. Considering that curative therapy has been available for Hodgkin's disease for more than 30 years, oncologists treating children and adolescents with the disease have an expectation of long-term survival for these patients. For many physicians, patients with Hodgkin's disease have been the "bright spot" in their practice because they are a group who uniformly respond well to therapy and overcome their disease. Unfortunately, long after their exit from paediatric practices, the true cost of curative therapy becomes readily apparent as aging survivors develop a variety of medical complications unquestionably predisposed by their antineoplastic therapy. Presently used regimens have several therapy related toxicities leading to significant late complications.<sup>35-37</sup> After 6 cycles of nitrogen mustard, vincristine, procarbazine, and prednisone (MOPP) there is usually persistent azoospermia in most male patients and there is a 3%-6% risk of secondary leukemias (mostly from nitrogen mustard). Six cycles of ABVD is associated with the risk of chronic cardiomyopathy (cumulative doxorubicin dose of 300 mg/m<sup>2</sup>) and impaired lung function with Bleomycin (cumulative dose of 120 mg/m<sup>2</sup>). Breast cancer is the most common solid tumor occurring in female Hodgkin's survivors with 94% of these tumors occurring in the irradiated field.<sup>36</sup> Current trials in paediatrics are therefore looking to decrease toxicity from chemotherapy and radiotherapy. The desire to prevent or reduce treatment sequelae, especially

second malignancies and cardiopulmonary dysfunction, has continued to motivate therapeutic modifications over the last several decades. While these complications adversely affect quality of life and increase the risk of early mortality, Hodgkin's disease remains the leading cause of death observed in several cohort studies of long-term paediatric survivors, underscoring the need to proceed cautiously with therapy refinements that do not compromise disease control.<sup>38,39</sup>

The study by Children's Cancer Group (CCG) trial designed to evaluate whether outcome of children with Hodgkin's disease treated with dose-intensive, multiagent chemotherapy is compromised by the omission of radiation.<sup>40</sup> is representative of many current risk-adapted paediatric Hodgkin's trials with the dual objectives of maintaining treatment efficacy while reducing late treatment complications. Long-term follow-up of childhood cancer survivors has permitted identification of specific clinical and treatment factors predisposing to the common sequelae of Hodgkin's disease. For example, breast cancer is almost exclusively observed in young women treated with thoracic radiation; treatment during puberty and higher cumulative radiation doses seem to enhance this risk.<sup>41-43</sup> With the expectation of long-term survival in 85% or more of children and adolescents who present with Hodgkin's disease, it is essential to consider clinical risk factors predisposing to late complications during treatment planning for newly diagnosed patients.

It is instructive to review the evolution of paediatric Hodgkin's therapy to appreciate the current treatment biases of many paediatric oncologists. In early treatment regimens, the patient's age or physical maturity was not considered during treatment planning. Standard-dose (35 to 44 Gy) radiation therapy to extended treatment volumes was the norm, for both children and adults, producing respectable disease-free survival rates for children with localized disease. However, long-term follow-up revealed treatment toxicity unique to children in the form of musculoskeletal growth inhibition.<sup>44</sup> A desire to avoid these deformities led to the development of treatment protocols specifically designed for children, which used low-dose, involved-field radiation and fewer cycles of non-

cross-resistant combination chemotherapy.<sup>45</sup> Standard-dose radiation therapy was subsequently reserved for older, skeletally mature patients with localized disease until concerns about radiation-induced cardiovascular disease and second malignancies eventually led to the abandonment of radiation as a primary treatment modality by most paediatric oncologists.<sup>41-43,46</sup>

Despite results from numerous paediatric trials supporting the efficacy of a combined-modality treatment approach, the desire to avoid radiation-related toxicity, particularly second malignancies, has motivated continued investigation of chemotherapy-alone treatment regimens.<sup>47</sup> Chemotherapy alone has long been established as an effective alternative to combined-modality therapy, but it confers risks associated with higher cumulative doses of anthracyclines, alkylating agents, and bleomycin.<sup>48</sup> This is particularly significant as early trials prescribed considerably more months (usually in the range of 8 to 12 months) of chemotherapy than is typically used today. Early chemotherapy trials used MOPP or similar regimens derived from MOPP. Chemotherapy-related acute toxicity was acceptable, but limited data on long-term treatment effects support the expected high incidence of gonadal toxicity.<sup>49</sup> Contemporary chemotherapy-only trials have used non-cross-resistant chemotherapy typically derived from MOPP and doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD). Early outcomes seem comparable with those achieved with combined-modality therapy, but long-term effects on cardiac, pulmonary, and gonadal function have not been reported.<sup>49</sup> Interpretation of these treatment results is further complicated by the fact that most of the studies comprised small numbers of clinically staged patients, assigned to treatments in a nonrandom fashion. In fact, some protocols specifically excluded patients with unfavorable features such as bulky or extensive lymphadenopathy, clinical features reported to benefit from a combined-modality treatment approach.

The Nachman et al<sup>40</sup> study joins the ranks of the relatively few prospective randomized trials evaluating treatment outcomes for paediatric Hodgkin's disease using chemotherapy alone compared with combined-modality therapy. Two

previous trials for advanced-stage paediatric Hodgkin's disease organized by North American cooperative groups failed to show a statistically significant advantage in event-free survival or overall survival with the addition of radiation therapy to non-cross-resistant chemotherapy.<sup>50,51</sup> The CCG compared 12 cycles of alternating MOPP/ABVD to six cycles of ABVD plus low-dose (21 Gy) radiation, and the Paediatric Oncology Group evaluated the benefit of adding low-dose radiation to eight cycles of alternating MOPP/ABVD.<sup>50</sup> The trend in event-free and overall survival, although not statistically significant, suggested an advantage for the combined-modality approach over chemotherapy alone for the CCG trial. For the Paediatric Oncology Group trial, the intent-to-treat analysis did not indicate an event-free or overall survival advantage for the group randomized to receive radiation after completion of eight cycles of alternating MOPP/ABVD, but the as-treated analysis showed superior outcomes for patients treated with combined-modality therapy.<sup>51</sup> The findings of both of these studies suffer from the fact that contemporary investigators have little desire to treat paediatric patients with 8 or 12 cycles of alternating MOPP/ABVD, with or without radiation therapy.

In the recent CCG trial reported by Nachman et al,<sup>40</sup> a contemporary chemotherapy regimen is prescribed, cyclophosphamide, vincristine, procarbazine, and prednisone/doxorubicin, bleomycin, and vinblastine (COPP/ABV), which substitutes cyclophosphamide for the more leukemogenic nitrogen mustard and compacts the traditional alternating non-cross-resistant chemotherapy combinations into a dose-intensive hybrid. This treatment approach offers the advantage of a reduced duration of therapy and lower cumulative doses of individual agents. For stages I through III, a risk-adapted treatment assignment was based on the presence of adverse clinical features such as hilar adenopathy, involvement of more than four nodal regions, bulky mediastinal (> 33% of chest diameter) or peripheral (more than 10 cm) lymphadenopathy, and "B" symptoms. Patients with favorable disease presentations received four COPP/ABV cycles, whereas patients with adverse disease features received six COPP/ABV cycles. Stage IV patients with extranodal disease received a more intensive therapy including

sequential cycles of high-dose cytarabine and etoposide, COPP/ABV, and cyclophosphamide, vincristine, doxorubicin, and methylprednisolone. Treatment was also response-based, as patients who achieved a complete response to initial chemotherapy were eligible either for randomization to receive low-dose involved-field radiation or no further treatment. The randomization was stopped earlier than anticipated because of results indicating a significantly higher number of relapses on the no-radiotherapy arm. The 3-year event-free survival estimates by both an intent-to-treat and as-treated analysis were significantly lower for patients treated with chemotherapy alone, with differences being most marked in stage IV patients. Because of successful salvage therapy for relapsed patients, estimates for overall survival are not different between the randomized groups in early follow-up. However, studies of long-term survivors from other series clearly indicate that treatment for relapse increases the risk of second malignancies and early mortality.<sup>37,38</sup> The authors conclude that chemotherapy alone is not as effective as combined-modality therapy, although they allude to preliminary data analyses suggesting that there may be a subset of patients in whom the likelihood of microscopic residual disease is small and who may benefit from treatment with chemotherapy. This information is critical to more accurately guide risk assessment and treatment assignment in the risk-adapted era.

Reaching consensus about the characteristics of the paediatric patient with Hodgkin's disease for whom therapy intensification is appropriate because of a high risk of treatment failure, or for whom outcome will not be compromised by further therapy reductions, has often been difficult to accomplish. For many trials, adverse prognostic features have included advanced (stage IIIB or IV) or unfavorable (bulky, symptomatic) disease presentations. To date, prognostic factor analyses in paediatric trials have revealed various findings related to laboratory parameters and tumor histology that have not yet been used to direct therapy. Likewise, only a few studies have correlated treatment outcome with biologic tumor activity, eg, interleukin-2 receptor elevation, but these features have not been studied prospectively to delineate their relationship with disease response

and long-term outcome.<sup>52</sup> More recent trials have demonstrated the prognostic significance of rapid early response, a paradigm that will be tested by upcoming Children's Oncology Group Hodgkin's trials.<sup>53</sup> Clearly, future progress in therapy for paediatric Hodgkin's disease will require an improved understanding of the clinical and biologic features that contribute to pathogenesis and treatment response. Until this information is available, paediatric investigators will persist with their careful manipulations of therapy in an effort to improve disease control and reduce long-term complications for children and adolescents with Hodgkin's disease.

### Late Effects

Children with Hodgkin's disease require long-term follow up. Cardiac function should be followed using echocardiography or MUGA scans since cardiac dysfunction can appear several years after anthracycline therapy<sup>54</sup>. Pulmonary function including diffusion capacity should be monitored following bleomycin use<sup>55</sup>. Thyroid profile should be followed closely since an asymptomatic patient with an elevated TSH is an indication to start thyroid replacement therapy<sup>56</sup>. Skeletal growth measurements with growth charts should be maintained and sexual maturation should be monitored. Screening for second malignancy<sup>57</sup> is necessary and in female patients, breast examinations and consideration for an early mammogram are required, if the breast tissue was in the irradiated field<sup>58</sup>. Historically, treatment has involved the use of procarbazine and alkylating agents such as chlorambucil, mustine and cyclophosphamide. However, whilst this treatment leads to good survival rates, the majority of male patients have subsequently developed permanent azoospermia<sup>59</sup>. Mackie *et al*<sup>60</sup> studied children who had received treatment with 'ChIVPP', a chemotherapy regimen containing chlorambucil and procarbazine. The mean age at diagnosis of the male patients investigated was 12.2 years, and of these 89% subsequently had evidence of severe damage to the seminiferous epithelium up to ten years following therapy. This study demonstrates both the gonadotoxicity of these agents, and also the susceptibility of the prepubertal testis. Whitehead *et al*<sup>61</sup> similarly followed up children treated with 'MOPP' chemotherapy, a regimen containing mustine

and procarbazine, and also demonstrated subsequent long-term testicular damage in the majority of male patients. In view of these studies, treatment for Hodgkin's lymphoma has been modified in an attempt to reduce the gonadotoxicity, whilst maintaining long-term survival<sup>62</sup>. Semen cryopreservation should always be considered for young men undergoing treatment for Hodgkin's lymphoma with potentially gonadotoxic treatment, fertility preservation in the female for those at risk of a truncated window of opportunity for fertility is more complex<sup>63</sup>.

### Treatment Of Refractory Or Relapsed Hodgkin's Lymphoma

Treatment outcome for patients with Hodgkin's lymphoma has steadily improved over the last half-century. Whereas a patient treated in the 1960s had an 80% chance of subsequent progression of disease, one treated in the 1990s has less than a 20% chance of developing the same problem. The need to have a strategy for the treatment of Hodgkin's lymphoma not cured by primary treatment remains important. Very few patients with limited stage Hodgkin's lymphoma demonstrate refractory or relapsed disease. Thus, a need to find effective secondary treatment for Hodgkin's lymphoma is confined almost entirely to patients presenting with advanced stage lymphoma.

High dose chemotherapy and irradiation plus autologous hematopoietic stem cell transplantation (HDC/ HSCT) has, over the past two decades, become established as the most effective treatment for patients whose Hodgkin's lymphoma has proven incurable with standard chemotherapy and radiation. Phase II trials, collected series from bone marrow transplantation registries<sup>64</sup> and two Phase III randomized trials<sup>65,66</sup> have demonstrated that the effectiveness of HDC/HSCT is sufficiently clear that HDC/HSCT has become widely accepted as the best treatment approach for most patients who are not cured by primary treatment programs based on multi-agent chemotherapy.

The high levels of toxicity and cost associated with HDC/HSCT demand that it be reserved for patients where it clearly increases the chance of cure compared to alternative treatments. This describes two groups of patients: first, those

whose disease progresses during primary chemotherapy or fails to enter a complete remission as proven by biopsy demonstrating persistent disease; second, patients who relapse after completing a full course of multi-agent chemotherapy with or without radiation. The first group, usually referred to as having refractory or chemotherapy resistant disease, has very little chance of cure with any program of standard dose chemotherapy with or without irradiation.<sup>67-</sup>  
<sup>69</sup> This group, lacking reliably curative alternatives, is best treated with HDC/HSCT because it offers a definite chance of cure. The use of HDC/HSCT for patients in first relapse after primary chemotherapy is somewhat more controversial, especially if the relapse occurs long after completion of the primary treatment or in an isolated nodal area easily amenable to irradiation. However, when relapse occurs after primary chemotherapy consisting of a regimen as effective as ABVD, the chance of inducing long-term disease-free survival with standard dose chemotherapy is small, probably less than 20 percent.<sup>67,68,70</sup>

Two special subgroups may not share this poor prognosis: those who relapse solely in originally involved but unirradiated lymph node groups;<sup>71-</sup>  
<sup>74</sup> and those who relapse more than 1 year after completion of the primary chemotherapy.<sup>67,68,75</sup> In the first of these 2 subgroups, wide field irradiation with or without additional chemotherapy may cure 40% to 50% of very carefully selected patients.<sup>71-74</sup> However, very few patients fit the ideal pattern of having nonbulky disease confined to lymph nodes at diagnosis and relapse, absence of B-symptoms at diagnosis and relapse and, preferably, a long interval from primary treatment to time of relapse. Although those relapsing more than a year after completion of primary chemotherapy may do well with a switch to potentially noncross-resistant chemotherapy with or without irradiation, this approach will only cure 20% to 40% of these specially selected patients.<sup>67,68,75</sup> In contrast, however, this same subgroup is the one with the very best outcome with HDC/HSCT.

In theory, the use of allogeneic stem cells, with their potential to add an immunologic attack on the malignant cells and provide a stem cell source free of contaminating tumor cells, should be even more effective than autologous stem cell

transplantation following HDC for Hodgkin's lymphoma. However, this improved potency is more than offset by increased toxicity leaving no net gain for the patient.<sup>87,88</sup> Any gain in disease control is overshadowed by increased toxicity, often lethal, from graft versus host disease and interstitial pneumonitis. Presently, with the availability of peripheral blood stem cells that appear to be free of clonogenic tumor cells and their proven efficacy and lower toxicity, autologous stem cells are the source of choice for hematologic engraftment when HDC/HSCT is used for Hodgkin's lymphoma.

Although most of the initial experience employing HDC/HSCT for Hodgkin's lymphoma was acquired using autologous bone marrow cells, most groups now use autologous peripheral blood stem cells.<sup>76-78</sup> In addition, most groups currently employ at least some standard dose chemotherapy prior to the high-dose chemotherapy for two reasons. First, it brings the Hodgkin's lymphoma under control while the logistics of stem cell collection and the hospitalization for HDC/HSCT are arranged.

Second, it provides priming for the peripheral blood stem cell collection enhancing the effectiveness of hematopoietic stem cells. However, it is important to remember that the purpose of this pre HDC/HSCT chemotherapy is not to test for chemosensitivity. Hodgkin's lymphoma, almost uniquely among human neoplasms, can be cured with the use of HDC/HSCT even when the disease does not respond to standard dose chemotherapy.<sup>79</sup>

Although a variety of HDC regimens have been described, no one regimen has been shown to be clearly superior. Currently, popular regimens include CBV (cyclophosphamide, carmustine [BCNU] and etoposide [ETOP]),<sup>64,80-83</sup> BEAM (carmustine [BCNU], etoposide, cytarabine and melphalan)<sup>65,84</sup> or high-dose melphalan with or without total body irradiation<sup>65,86</sup> Because none of these regimens has been shown to be superior, it is more important for investigators at an individual center to master the management of the acute and chronic toxicities of their chosen regimen than to switch from one to another seeking some modest but unproved advantage.

### Newer Therapies For Hodgkin's Disease

Most series suggest 6 % of patients still dying of

progressive lymphoma despite optimal use of primary chemotherapy and secondary HDC/HSCT there is a clear need to find effective new therapeutic agents. Gemcitabine is the most promising traditional type chemotherapeutic agent currently under investigation for Hodgkin's lymphoma.<sup>89-94</sup> In small series of heavily treated patients, an overall response rate of approximately 50% has been found with 10%–20% complete responses. Even more encouraging, 2 groups have found an overall response rate higher than 75% when gemcitabine was combined with cisplatin and a corticosteroid.<sup>89,90</sup> This promising new agent will need further testing and integration into combinations with standard or other novel agents to exert its ultimate impact in the management of Hodgkin's lymphoma.

One of the most promising new types of treatment for lymphoma, in general, is targeted immunotherapy. The anti-CD20 monoclonal antibody rituximab has proven useful for several different types of B cell lymphomas. The nearly universal expression of CD20 on the neoplastic cells of LPHL suggests rituximab may be useful. Preliminary data from several small series show response rates exceeding 50%<sup>91-95</sup> however, the durability of these responses seems limited. Treatment with rituximab is attractive for this disease because of the lack of cumulative or late toxicity with this agent but will need to be integrated with conventional treatments to have a substantial impact. Efficacy of one type of targeted immunotherapy hints that others may also be useful. Monoclonal antibodies aimed at other B cell or lymphocytic antigens,<sup>96</sup> and immunotoxin molecules<sup>96-100</sup> including bispecific antibodies and, eventually, tumor specific immunization strategies<sup>101</sup> all hold promise. New immunotherapeutic approaches such as these hold substantial promise for Hodgkin's lymphoma. However, because this disease is already so often cured, finding subjects for the testing of new agents is increasingly difficult. The challenge for clinicians involved in the treatment of Hodgkin's lymphoma is to maintain the high cure rates but decrease the long-term morbidity, particularly second tumours, cardiac dysfunction and infertility. Combined modality treatment has been the gold standard for three decades and it remains to be seen whether functional imaging

in the form of FDG-PET can determine which patients need combined modality treatment and which will be cured by chemotherapy alone.

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# Achievements and Future Perspectives in the Treatment of Multisystem Langerhans Cell Histiocytosis

Helmut Gadner & M. Arico

## Clinical stratification and response evaluation

The ignorance of the pathogenesis and the failure to establish generally accepted diagnostic criteria have inhibited the development of a rational treatment policy for multisystem LCH. The therapy for LCH, therefore, has varied over the past century according to what was believed to be the cause of the disease. Only with the introduction of new concepts of staging and diagnostic criteria it became possible to collect large enough numbers of patients to carry out prospective clinical trials.<sup>1</sup> Empirically it has been shown that the treatment of LCH should depend on the extent of the disease, and patients were stratified as having “single system” disease (bone, skin, lymphnode, lung, or CNS) with single site and multi site involvement, and “multisystem” disease, often associated with dysfunction of the so called “risk organs”, i.e. liver, lungs, spleen or hematopoietic system.

As the disease may follow different natural courses a new definition and assessment of response to a given treatment had to be established. The following criteria were defined by the Histiocyte Society in 1990:

- complete resolution of the disease (no active disease, NAD),
- disease regression (active disease, AD-better),
- intermediate response with regression of some and reappearance of other lesions (AD-intermediate, mixed) or unchanged disease (AD-intermediate, stable), and
- progression of the disease (AD-worse)<sup>2,3</sup>

## Prospective clinical trials in multisystem disease (MS)

Two major approaches existed throughout the last twenty years for the therapy of multisystem disease. They included a

conservative approach (single center study) with treatment used only during disease exacerbation,<sup>4</sup> and an approach with intensive chemotherapy induction followed by continuation treatment (carried out by two large cooperative clinical trials: the Italian AIEOP-CNR-HX 83 study<sup>5</sup> and the German/Austrian DAL-HX 83/90 study<sup>6</sup>) Despite different strategies, the overall mortality was about 20% in both therapy approaches. In contrast, the incidence of disease-related permanent consequences (late sequelae) was 67% in the conservative treatment study and only 33% in the DAL-HX studies. The comparison of the study results evidenced a lower incidence of disease reactivations in the DAL-HX studies (overall 23%) indicating that effective treatment may beneficially influence the natural course of the disease.

In the first international randomized chemotherapy trial LCH I, initiated by the Histiocyte Society in 1991, the efficacy of monotherapy with vinblastine and etoposide regarding the course of disease and outcome was compared in a randomized way.<sup>3</sup> No significant difference was found with respect to early and late response to treatment as well as prevention of recurrences and late sequelae. In the following international trial LCH II continuous oral prednisone combined with vinblastine with or without the addition of etoposide was compared, adopting a new stratification system to distinguish between “risk” patients with involvement of “risk organs” like liver, spleen, lungs, hematopoietic system or age under 2 years, and “low risk” patients without such organs involved and age beyond 2 years.<sup>2</sup> “Risk” patients were randomized between arm A (2-drugs) and arm B (3-drugs), “low risk” patients uniformly received initial treatment according to the 2-drug arm only. All patients received continuation therapy with 6-mercaptopurine and prednisone/vinblastine pulses. The whole

treatment duration was limited to 24 weeks as it was in LCH I.

In the “low risk” group 89% of patients were responders at the week 6 evaluation, and no fatalities occurred. Among the “risk” patients, the overall response rate was superior to that in the LCH I study and similar to that of the previous DAL studies. Between arm A and B in “risk” patients, however, no statistical difference with respect to response, survival and reactivation free survival has been found. Patients with involvement of “risk organs”, who did not show disease regression by week 6 or 12 of therapy, had a poor outcome and a high rate of mortality (approximately 20%). This figure did not differ from that in the DAL and LCH I studies. Notably, all patients who died in LCH I and LCH II studies irrespectively of age had at least one “risk organ” involved at diagnosis. It seems justified, therefore, to regard “risk organ” involvement and response to initial treatment as the most important prognostic factors, whereas age under 2 years did not prove to be of independent prognostic importance. Consequently, non responding patients might benefit from a rapid switch to an alternative salvage treatment.

The probability of reactivation within two years after complete response (non active disease) was about 50% in both low risk and risk patients. This compared well with the results in the LCH I study, but was inferior to the results of the DAL studies, which demonstrates a potential benefit of a longer treatment duration (12 months in DAL studies vs. 6 months in LCH studies).<sup>7</sup>

In the ongoing third international randomized trial LCH III ([www.histiocyte.org](http://www.histiocyte.org)) patients are stratified into three groups:

- (1) multisystem patients with involvement of one or more risk organs (“risk” patients),
- (2) multisystem patients without involvement of risk organs (“low risk” patients), and
- (3) single system patients with “multifocal bone disease” or localized “special site” involvement (paranasal, parameningeal, periorbital, and mastoid region or intraspinal extension) which can lead to persisting soft tissue swelling.

These locations are considered to be risk sites

for CNS disease, except surgical excision is feasible (e.g. periorbital eosinophilic granuloma).<sup>8,9</sup> “Risk” patients after central randomisation are entered in two different treatment arms (arm A and B) which include prednisone, vinblastine and 6-mercaptopurin (6-MP) with or without methotrexate (MTX). Initial treatment consists of one or two courses depending on response and is followed by continuation therapy with 6-MP/MTX and Pred/VBL pulses (treatment duration 12 months). For “low risk” patients a standard therapy with prednisone and vinblastine is recommended with random assignment to a continuation therapy (6-MP/MTX and pulses) for 6 or 12 months. Patients with multifocal bone or special site involvement are treated with prednisone and vinblastine followed pulse therapy (Pred/VBL) (treatment duration 6 months). So far, the overall results in the two risk groups are comparable with the outcome in the LCH II study with respect to response and mortality. It is too early to communicate further more detailed information regarding the impact of MTX in arm B and treatment duration on the reactivation frequency and rate of permanent consequences.

### **New approaches for resistant disease**

Not responding “risk” patients are considered to have a high risk of mortality (about 75%). Cyclosporin A alone and in combination with dexamethason and anti-thymocyte globulin has been suggested as an alternative treatment approach, however, especially in patients with advanced chemotherapy-resistant multisystem disease convincing data of efficacy is lacking.<sup>10,11</sup> Also regarding the role of bone marrow transplantation only few and inconsistent data is available. Especially, myeloablative stem cell transplantation as a possible salvage approach for these patients has shown to be associated with a high risk of transplant-related mortality (45%). Therefore, allogeneic stem cell transplantation following a reduced intensity conditioning regimen (RIC-SCT) has been performed recently, as an alternative salvage approach with promising preliminary results. Seven out of nine patients with persistent risk organ involvement survived and were in good clinical condition after a median follow-up of 390 days post transplantation.<sup>12</sup> Notably, even in

those patients who responded well to RIC-SCT, clinical recovery from the underlying disease after transplantation was slow and protracted, reflecting a slow and gradual decrease of cytokine load and related symptoms, which seem to be a peculiarity for this disease. These data underline the potential utility of RIC-SCT for LCH patients with resistant “risk organ” involvement.

The use of 2-chlorodeoxyadenosine (2-CDA) has recently appeared to be successful in refractory LCH.<sup>13</sup> According to the (recently closed) salvage treatment protocol of the Histiocyte Society, 2-CDA was given as a monotherapy (5 mg/m<sup>2</sup> 2-CDA daily for 5 days at 3-4 weekly intervals; 2, 4 or 6 courses) to non-responding multisystem patients or patients with recurrent disease. The overall response rate was disappointing in therapy-resistant high risk patients. However, patients with recurrent disease manifestations in non-risk organs showed a similar good response rate as those who obtained standard Pred/VBL combination, but the risk of disease reactivation after stopping therapy was equally high as it was with standard therapy. Recently, the results of a pilot study of 2-CDA and Ara-C combined chemotherapy were published by the French LCH Study Group.<sup>14</sup> Ten children with refractory LCH and severe hematological dysfunction, median age of 0.5 years at diagnosis, received at least 2 courses of ARA-C (1000 mg/m<sup>2</sup>/d) and 2-CDA (9mg/m<sup>2</sup>/d) administered during 5 days every 4 weeks. Seven out of these very high risk patients survived with resolution of disease after a median follow-up of 2,8 years. The encouraging results of this study together with the new experiences with RIC-SCT are a matter of debate and consideration within the Histiocyte Society for further investigation in a prospective way.

Other studies have reported success in the treatment of LCH with interferon- $\alpha$ , thalidomide, anti-CD1a, and tumor necrosis factor- $\alpha$  antibodies.<sup>2,15,16</sup> Anecdotal experience has been published recently, regarding the use of imatinib mesylate and cladribine in CNS disease.<sup>17,18</sup> Each of these approaches clearly needs to be evaluated prospectively before they can be recommended as standard for non-responsive MS-LCH.

### How recurrences can be prevented?

A major problem in the treatment of MS-LCH is the prevention of recurrences. The probability of reactivation within two years after complete response (non active disease with resolution of all symptoms and signs) is in the range of 50% in both “low risk” and “risk” groups of the LCH I and LCH II studies. In both of these studies the treatment duration was only 6 months. Interestingly, in the DAL studies after one year therapy the reactivation rate was lower, suggesting a potential benefit of longer treatment.<sup>7</sup> Reactivation presents usually with bone and/or skin involvement and can easily be controlled by standard therapy. A recurrence in “risk organs” after NAD is only rarely observed. Many attempts have been undertaken to find a strategy to prevent reactivations. This objective is going to be addressed by the LCH III study with the prolongation of the whole treatment period to 12 months in “risk” patients, and a randomized assignment of patients with “low risk” features to a 6 and 12 months therapy, respectively. The first reactivation mostly occurs after stopping therapy until 2 - 3 years later. Further reactivations may be seen within the same time period as after first line therapy in approximately one third of children, declining from year to year. In a cohort of 563 patients with LCH the reactivation frequency vanished after 5 years of follow-up to nearly zero, which indicates that the disease is self-limited (unpublished results of the LCH studies, Vienna). So far, no difference has been seen also after second line therapy with 2-CDA or other drugs.

The Salvage Therapy Study Group is trying hard to set up an appropriate treatment protocol for patients with recurrent disease and is looking for broad cooperation.

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# Soft Tissue Sarcoma – Current Management

Meriel Jenney, Ken Brown

**T**he treatment of soft tissue tumours (STS) in children and adolescents is complex. The commonest STS occurring in childhood is rhabdomyosarcoma (RMS) and we will concentrate on its management in this presentation. However the complexity of other STS that occur more commonly in older children and adolescents, particularly issues of local control will also be reviewed.

The challenges of the treatment of STS in childhood

- STS in childhood are rare. There are many pathologies, although RMS is the commonest in childhood. Even for this tumour, treatment depends many variables, including the age of the child, the site, stage and size ( $> / < = 5\text{cm}$ ) of the tumour as well as the pathological subtype (alveolar, embryonal) and clinical (post operative) IRS grouping.
- There is a need for collaborative, multimodal approach and treatment dependent on many factors. Treatment of the tumour is often complex, depending on good 'local control', i.e. surgery and radiotherapy and appropriate systemic therapy (chemotherapy).

## Progress has been made

- The understanding of the biology (cyto- and molecular genetics) of the tumour has improved. The identification of the translocation in alveolar RMS has been important, particularly in the identification of those patients who have tumours with a poorer outlook. The  $t(2;13)$  and  $t(1;13)$  are known to be associated with alveolar disease the former carrying the poorer prognosis, the latter having a prognosis more like that of embryonal disease. The fusion of the PAX 3 and PAX 7 with FKHR has also been demonstrated to occur as a result of these translocations (Barr, Sorensen), allowing for

even more sensitive identification of poor risk tumours.

- Risk stratification for RMS is improving. There is a need to optimise local control and, as overall survival rates improve, there aim is to not to over- or under-treat patients wherever possible. Risk stratification in RMS is complex. Historically there have been different approaches between European and North American countries to both risk stratification and therapy. Understanding these differences and their impact on event free survival, overall survival and late effects improves our understanding of the disease. Whilst the overall survival improves there is still more to learn about which patients are at higher risk, requiring systematic local therapy i.e. radiotherapy (e.g. those with parameningeal rhabdomyosarcoma) and which can potentially be cured without local therapy (e.g. some group II patients and even those with Group III tumours at particularly 'good' sites e.g. the orbit (Oberlin), in addition to many group I patients. It is clear that there are differences in outcome between the recent Intergroup Rhabdomyosarcoma Study (IRS IV) (*Crist*) and the SIOP study MMT 89 (*Stevens*), with better EFS and OS for some groups of patients treated within the IRS study, conversely there are some in the MMT study cured without the need for radiotherapy with its late sequelae, particularly important for those who receive therapy at a very young age.
- For patients with non –RMS STS, there is greater understanding of pathological grading with comparisons between the French FNCLCC and POG classification undertaken. It is hoped that further understanding of the molecular and cytogenetics these tumours (e.g. the  $t(12;22)$  translocation in clear cell sarcoma) of will soon follow.

### Improvements in the delivery of local therapy

The three major risk factors of prognostic significance in soft tissue sarcomas are tumour grade, size and depth (Wunder et al., 2000). In rhabdomyosarcoma (RMS) nodal status is also very important. In this disease regional nodal involvement is not considered metastatic disease while in other soft tissue sarcomas it is. Enneking et al 1980, described a schema for classifying surgical margins which were radical, wide, marginal and intra-lesional. Essentially there are only three types of surgical procedures which are utilized for soft tissue sarcomas. They are: intralesional (R2), marginal R1) and wide (R0) excisions. The corresponding pathologic margins are gross residual, microscopic residual and no residual (clear) margins. A wide margin (R0) is achieved when the tumour is removed en bloc and the plane of dissection is through a narrow rim of normal tissue surrounding the pseudocapsule and reactive zone of the enclosed tumour. Currently a margin of normal tissue 5mm thick or an intact fascial layer is considered the minimal acceptable wide margin. In a marginal excision (R1), the tumour is removed en bloc but the plane of dissection cuts through the tumour pseudocapsule or reactive area surrounding the tumour leaving microscopic residual disease. For an intra-lesional margin (R2), the tumour is exposed during surgery and gross macroscopic tumour is left behind.

#### Types of Surgical Procedures:

Surgical Procedure		Pathologic Results
Wide Excision	R0	Clear Margin
Marginal Excision	R1	Microscopic Residual
Intralesional Excision	R2	Gross residual

The adequacy of the margin of resection is the most important variable that is associated with local relapse (Bell et al., 1989; Baldini et al., 1999; Spiro et al., 1997) while tumour size is the major determinant of the risk of systemic disease (Bell et al., 1989). Data from the IRSG (Intergroup Rhabdomyosarcoma Study Group) indicates the most frequent sites of primary tumor to be the genitourinary tract (23%), extremity (17%), parameningeal area

(nasopharynx, nasal cavity, paranasal sinuses, middle ear-mastoid area, pterygopalatine, and infra-temporal fossa (16%), other head and neck sites (excluding orbit) (10%) and orbit (8%). The tumor also arises in the trunk (10%), retroperitoneum (11%), and miscellaneous other sites (Maurer et al., 1993).

Rhabdomyosarcomas are very sensitive to radiotherapy and chemotherapy and in many sites such as the orbit, parameningeal sites and some sites of the genitourinary tract, this is the mainstay of local therapy particularly when the tumour is of embryonal subtype. Some patients with wide excisions (R0) of tumors of non-alveolar histology do not require routine postoperative radiation therapy. Extremities are unfavorable sites for rhabdomyosarcoma partly due to the high (80%) incidence of alveolar histology and the large size >5cm of many of the tumors. Regional nodes are involved in about 17% of tumors arising in an extremity site. (Neville et al., 2000) Nodal involvement is a poor prognostic indicator. Recent publications have highlighted the differences in therapy between some European centers and those in North America (Donaldson et al., 2001; Stevens et al., 2005). The North American approach has been to increase the radiation dose and the number of patients receiving radiation therapy to improve local control while in Europe selected patients with local recurrence are given additional therapy to return the patients to a better overall survival. Despite the differences in local relapse, overall survival is surprisingly similar in the two groups. Future collaborative studies may clarify which approach is best (Donaldson, Anderson, 2005; Stevens, 2005).

The therapeutic approach in other types of soft tissue sarcomas is quite different. There is a paucity of good studies in children and most information comes from the abundant adult literature. In non rhabdomyosarcoma soft tissue sarcomas surgery is the mainstay of therapy and radiotherapy forms an adjunctive role. Radiotherapy alone has a poor outcome with only about 1/3 of patients obtaining control of disease (Slater et al., 1986; Tepper, Suit, 1985). A randomized study by Yang et al illustrated the benefits of adjuvant radiotherapy for local control. Twenty two percent of patients with high grade large tumours recurred without

radiotherapy but when a wide margin (R0) was obtained there were no local recurrences (*Yang et al., 1998*). This demonstrates the necessity of obtaining clear pathologic margins to obtain adequate local control. Radiotherapy can be given either preoperatively or postoperatively. Pre operative radiotherapy has the disadvantage of a higher incidence of wound complications following surgery but has the advantage of treating a smaller volume of tissue and possibly administering a smaller dose. (*O'Sullivan et al., 2002*). Radiotherapy has the complication of tissue oedema and fibrosis and decreased range of joint motion (*Davis et al., 2002; Davis et al., 1999*). There is also a risk of development of a radiation induced sarcoma. All of these complications are dose dependant. In children there is the added complication of future limb length discrepancy due to growth arrest of the physeal bone growth at the ends of the bone. The irradiation field size in children may be smaller with preoperative radiotherapy since the postoperative wound does not require treatment. In some cases this may allow growth plate sparing. In the future efforts will be made to diminish late effects while preserving good local control. Modalities such as dose reduction, brachytherapy or Intensity Modulated Radiation Therapy (IMRT) are being investigated in some centres.

Unplanned surgical excisions (UPS procedures) are problematic since there is no preoperative staging imaging to know initial tumour extent or the lesion has been excised without an adequate cuff of normal tissue surrounding the tumour. In these patients there has been an intra-lesional excision (R2) with a wide contamination of the operative field. Further local treatment to treat the problem is more extensive than would have been necessary if the proper staging and surgery was done. This leads to worse functional outcomes and a greater risk of local recurrence. (*Davis et al., 1997; Noria et al., 2002*)

#### Where next?

- Whilst the majority of clinical trials have centred around optimising chemotherapy in RMS, little progress has been made. Vincristine (V), Actinomycin D (A) and Cyclophosphamide (Cyclo) remain the gold standard of therapy in the Children's Oncology Group (COG) studies, whilst therapy VA with Ifosfamide (Ifos) in Europe. The efficacy of Cyclophosphamide and Ifosfamide in RMS appears to be equivalent and the choice of alkylating agent depends on the accepting the different toxicity profile (impairment of fertility with cyclo versus encephalopathy (reversible) and nephrotoxicity with ifos). Costs also differ with drugs costs and the cost of administration being greater for Ifos.
- The role of anthracyclines is debated and although it has not been proved in the phase III setting, a previous IRS II window study and a recent SIOP window study (Bergeron personal communication) have shown excellent partial and complete remission rates. Doxorubicin will be administered in a more dose intensive schedule in the new European non metastatic RMS study, in a randomised setting. Vinorelbine has been demonstrated as active in phase II studies in alveolar rhabdomyosarcoma (*Casanova*) this agent will also be tested in the phase III setting.
- Positron Emission Tomography (PET), particularly combined with CT or MRI appears to be of value in non- RMS STS. Of particular value is the ability to quantify tumour activity (SUV) and to identify active areas for biopsy. Fusion techniques may also be of value when planning definitive surgical procedures. The role of PET in RMS has yet to be established.

#### Where are the problems in the management of RMS and other STS?

- The treatment of metastatic disease remains a real challenge with very little improvement in survival for patients with poor risk, metastatic tumours. The relative rarity of this (and other) subgroups has made randomised studies almost impossible. However future strategies evaluating the role of high dose therapy and maintenance chemotherapy are proposed.
- Optimising local control is also critical. Identifying those who can be cured without radiotherapy or major surgery or conversely optimising surgery/ radiotherapy/ brachytherapy for patients who have tumours

with a high risk of recurrence remains a high priority within current trials. A particular problem is bringing together the appropriate specialists with the skills to manage tumours at difficult sites, often in very young children.

- The lack of new agents over recent years has limited progress although Irinotecan in combination with vincristine will be brought forward into the new phase III COG study (IRS VI) having been demonstrated as highly active in the window setting (*Pappo*). The role of agents with anti-angiogenesis properties is currently being explored and may also be an exciting way forward in the treatment of these challenging tumours.

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# Germ Cell Tumors in Children and Adolescents

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## Introduction

Germ cell tumors (GCTs) constitute a highly heterogeneous group of tumors that significantly varies with respect to site, clinical presentation, histology and biology. This heterogeneous clinical presentation requires a multimodal treatment that includes the paediatric oncologist in cooperation with the appropriate surgical disciplines (paediatric surgeon, urologist, gynecologist, thoracic surgeon, and neurosurgeon) and the radiotherapist. During the past two decades, a dramatic improvement of the prognosis of malignant GCTs both in the adult and in the paediatric population has been achieved. This progress can mainly be attributed to national and international cooperative therapeutic protocols that utilized cisplatin-based combination chemotherapy as part of a multimodal therapeutic approach. The following chapters summarize the rapid development during recent years, and describe what should be considered up-to-date therapy of paediatric GCT.

## Epidemiology

Germ cell tumors may become clinically apparent in all age groups, ranging from the fetal period to adulthood. Among children younger than 15 years, GCTs are comparably rare and account for approximately 3-4% of all diagnoses enrolled onto epidemiologic registries. During childhood, the majority of GCTs present at nongonadal sites close to the body axis, e.g. the sacrococcygeal region, mediastinum or the pineal gland. Two incidence peaks can be observed within the paediatric tumors. The first peak includes teratomas (in neonates) and yolk sac tumors (during infancy and early childhood) that predominantly arise in the sacrococcygeal region, testis and less frequently the mediastinum or retroperitoneum.

In the second decade of life another rise of incidence can be seen that is due to the gonadal, mediastinal and CNS tumors. Histologically, these tumors resemble germinoma (syn.: seminoma, dysgerminoma) or nonseminomas including embryonal carcinoma, choriocarcinoma and yolk sac tumor. Ovarian cystic teratoma (dermoid cyst) most commonly occur during adolescence and comprise a distinct clinical entity that is characterized by a specific biology and benign clinical behavior.

## Histologic Classification of Germ Cell Tumors

GCT are characterized by a profound heterogeneity of their histologic differentiation. They are classified according to the WHO-classification of testicular, ovarian and intracranial tumors respectively (**Table 1**). As intra-tumor heterogeneity may be subtle, the initial diagnostic work-up should include the evaluation by a pathologist experienced in GCT histology in order to achieve a standardized and reliable histopathologic diagnosis and grading (**Table 1**).

According to the holistic concept of Teilum GCT arise from totipotent primordial germ cells which are capable of embryonic and extraembryonic differentiation. In contrast to testicular GCT of adult patients paediatric GCT do not develop from carcinoma *in situ*.

In most patients, the response to the different therapeutic modalities can be predicted from the histologic appearance and the tumor marker profile (**Table 2**). About 25% of all paediatric GCT present as tumors with more than one histologic type. In this situation therapy and prognosis depend on the component with the highest malignancy <sup>(1)</sup>.

**Table 1: Histologic classification of germ cell tumors according to the World Health Organization (WHO)**

	Synonyms
1. Seminoma (SE)	Dysgerminoma (DYS, ovary), Germinoma (GE, brain)
2. Yolk sac tumor (YST)	Endodermal sinus tumor
3. Embryonal carcinoma (EC)	-
4. Choriocarcinoma (CHC)	-
5. Teratoma (TER)	
5.1. Mature	-
5.1.1. Solid	-
5.1.2. Cystic	Dermoid Cyst
5.2. Immature (IT)	-
5.3. with malignant transformation	-
5.4. Monodermal	-
6. Tumors with mixed histology (MGCT)	-
7. Spermatocytic Seminoma (SS)	
8. Polyembryoma (POLY)	-

**Table 2 : Biological characteristics of the histologic germ cell tumor subentities**

	Histological Grading	tumor marker		sensitivity to	
		AFP	β-HCG	chemo-therapy	radio-therapy
seminoma/germinoma	malignant	-	(+)	+++	>24 Gy
embryonal carcinoma	malignant	-	-	+++	>45 Gy
yolk sac tumor	malignant	+++	-	+++	>45 Gy
choriocarcinoma	malignant	-	+++	+++	>45 Gy
Teratoma, mature/immature	benign/ pot. malignant	-(+)	-	?	?

### Biology

Molecular studies of GCT revealed that gonadal and nongonadal GCT share a common cellular origin<sup>(2)</sup>. While no consistent correlation between cytogenetic aberration and primary site of the tumor has been observed, it is apparent that histology (teratoma vs. malignant GCT) and age (pre- vs. postpubertal) both significantly correlate with distinct genetic profiles<sup>(3)</sup>. More than 80% of malignant testicular GCTs of young males display a distinct and specific chromosomal aberration, the isochromosome 12p<sup>(4)</sup>. The remaining isochromosome 12p - negative

tumors frequently show amplification of 12p (homogeneously staining regions or tandem repeats), and candidate genes have recently been identified in this region.

In contrast to adult patients, in malignant GCT of children younger than 10 years an isochromosome 12p has rarely been found. On the other hand, aberrations at chromosomes 1, 6, and 20 and the sex chromosomes have been found frequently<sup>(4)</sup>.

Lastly, virtually all pure teratomas are cytogenetically normal. However, cystic teratoma

of the ovary may present with isodisomic karyotype, consistent with their origin from postmeiotic germ cells.

### Diagnosis

In general, GCTs tend to occur as indolent masses, and clinical symptoms are mostly related to local dysfunction by tumor growth. If a GCT is suspected on the basis of the clinical assessment, a defined program of clinical, radiographic, and laboratory investigations has to be followed in timely fashion. The radiographic procedures must be performed with respect to tumor site and potential ways of tumor dissemination.

### Tumor markers

Depending on their histologic differentiation GCT tend to secrete the tumor markers AFP and/or HCG/ $\beta$ -HCG (**Table 2**). These facilitate clinical diagnosis in tumors that present at typical sites <sup>(5)</sup>. It is important to note that in contrast to other reports, this series of normal neonates in infants has demonstrated that in a significant percentage of healthy children, the AFP does not decline to the normal range of adult patients before the end of the second year of live <sup>(6)</sup>. Therefore, in the first two years of life, only AFP levels significantly above the age-related normal value can be regarded as diagnostic for a secreting GCT.

In general, the tumor marker profile is highly specific for the histologic differentiation of the tumors (**Table 1**). In CNS GCT it is frequently observed that markers maybe elevated at different levels in CSF and serum. Therefore measurement at diagnosis in both compartments is mandatory.

About 20% of germinoma may secrete the Placenta-like Alkaline Phosphatase (PLAP), which can be used also as additional diagnostic tool, if elevated.

### Diagnostic imaging

In most patients with extracranial tumors, the initial radiographic assessment of the tumor will be made by ultrasound. During ultrasound, the tumor should be measured in three dimensions, and in addition, the abdomen and the lymph nodes should be screened for metastases. The next step will be to perform CT-, preferably MRI scans of the tumor.

### Laboratory studies (pre-treatment)

In addition to the tumor markers AFP and  $\beta$ -HCG, serum LDH has proven a prognostic marker in adult patients with GCTs. In germinomas, the Placenta-like Alkaline Phosphatase (PLAP) can be measured in the serum and may then serve as a marker of treatment response during follow-up. In addition to the routine blood tests and tumor markers specific attention should be given to the renal function (creatinine clearance, urine electrolytes), as several cytotoxic agents such as platinum-compounds and ifosfamide may interfere with renal function.

### Surgery

If the initial radiographic assessment uncovers infiltration into adjacent organs and/or metastases, up-front chemotherapy followed by delayed tumor resection is recommended, as preoperative chemotherapy will facilitate complete resection on delayed surgery <sup>(7)</sup>. For most sites except the liver and the retroperitoneum, tumor marker measurement in combination with imaging allows for a clinical diagnosis. In equivocal cases (i.e. non-diagnostic markers, hepatic or upper retroperitoneal tumors), a diagnostic biopsy is recommended.

If the radiographic assessment indicates a localized tumor without metastatic spread, a primary tumor resection constitutes the treatment of choice, except in CNS, as this region bears specific risks of surgical morbidity. In patients with tumor residues after initial tumor resection, 2<sup>nd</sup> look surgery is essential to achieve secondary complete resection. This is also the case in malignant non-germinomatous CNS GCTs. Finally, surgery of metastases is not indicated unless they show insufficient response to chemotherapy <sup>(8)</sup>.

### Cisplatinum-based Chemotherapy

The modern era of GCT chemotherapy began in the mid 1970s with the identification of the efficacy of cisplatinum in testicular GCT. In 1977, Einhorn and Donohue reported a complete response rate of 85% in patients with metastatic testicular GCT with a combination of cisplatinum, vinblastin and bleomycin (PVB) in adjunct to tumor resection <sup>(9)</sup>. Most importantly, in contrast to previously reported regimen with only vinblastin and

bleomycin, the overall good response was also translated into durable remissions.

Nevertheless, relapses or refractory cancers – although rare – established the need for second line therapies. Etoposide soon emerged as an active drug with a single-agent efficacy superior to vinblastin. On the other hand, the use of etoposide can be associated with therapy-related leukemias in approximately 1-2% of patients. In addition, the efficacy of ifosfamide in cisplatin-resistant GCT has been documented. The combination of cisplatin with etoposide and ifosfamide for recurrent testicular GCT results in a 30% durable remission rate and can now be considered standard relapse treatment. These observations have initiated studies that included etoposide and/or ifosfamide into the first line treatment of GCT.

In relapsing and refractory GCTs, the therapeutic value of high dose chemotherapy with autologous stem cell transplantation has been investigated. These analyses have shown only limited efficacy in prognostically unfavorable tumors such as cisplatin-resistant, mediastinal GCTs with high  $\beta$ -HCG are multiple relapses. Nevertheless, in some patients introduction of high dose chemotherapy into first-line treatment of high-risk tumors may be beneficial.

**Development of Cooperative Protocols for Paediatric GCTs**  
The first published trial was conducted by the US Childrens Cancer Group (CCG) and included 54 children with malignant nonseminomatous GCT. Patients underwent initial tumor resection followed VAC+PVB chemotherapy over a two year period, second look resection four months after diagnosis and irradiation in case of residual tumor. Fifteen of 20 evaluable patients with ovarian nonseminomatous GCT achieved a continuous clinical remission. The prognosis of children with nongonadal GCT was worse - ten of 18 evaluable patients achieved CR - but still encouraging compared to all other previous studies.

The analysis of the consecutive CCG protocol included 93 children. Patients with ovarian GCTs had a better prognosis (4 year EFS 63%) than children with nongonadal GCTs (4 year EFS 42%)<sup>(10)</sup>. This difference was mainly attributed to a higher rate of incomplete tumor resections in nongonadal tumors.

In the consecutive US Intergroup protocol, a watch-and-wait policy was followed in stage I testicular GCTs. Intermediate risk patients (testicular stage II, ovarian and nongonadal stage I-II) received four cycles cisplatin, etoposide, and bleomycin (reduced to one infusion per cycle compared to three infusions in corresponding adult regimens). Furthermore, in high risk patients (stage III-IV), the therapeutic impact of cisplatin dose intensification at 200 mg/m<sup>2</sup>/cycle was evaluated. The analysis of both gonadal and nongonadal GCTs revealed that higher doses of cisplatin may result in higher response and complete remission rates (approximately 9 % benefit), however at a significantly higher renal and auditory toxicity. More recent investigations of this study group also stated that amifostine protection during cisplatin therapy at escalated doses gives no significant benefit with regard to ototoxicity has been demonstrated, and amifostine therapy was associated with significant electrolyte imbalances, particularly hypocalcaemia<sup>(11)</sup>.

The analysis of different chemotherapy regimens administered in the British UKCCG GC I and GC II protocols also demonstrated the high therapeutic efficacy of platinum-based regimens such as BEP or JEB (carboplatin (600 mg/m<sup>2</sup>/cycle), etoposide, bleomycin) that resulted in a five year EFS of 57% and 87% in nongonadal GCTs<sup>(12)</sup>. The recent analysis of the UKCCG GC II study underscores the high efficacy of the JEB regimen, that resulted in a 5-year EFS of 88% with a favorable toxicity profile<sup>(13)</sup>.

The French study group reported 35 children with ovarian and nongonadal advanced stage GCTs that were treated with a VAC+PB regimen, and a two year survival of 63% was achieved<sup>(14)</sup>. The French cooperative protocol TGM 85 used a similar chemotherapeutic approach, and in the consecutive TGM 90 protocol, cisplatin was replaced by carboplatin (400 mg/m<sup>2</sup>/cycle)<sup>(15)</sup>. The results were less favorable with this regimen than with the British JEB regimen. This difference was mainly attributed to the lower single and cumulative dose of carboplatin. In the recent French protocol, alternating combinations of cisplatin with etoposide or ifosfamide are administered, resulting in a superior response rate compared to the previous carboplatin based strategy.

In both the French TGM 90 and the British GC II studies, the analysis of prognostic factors revealed the prognostic impact of high AFP serum levels at diagnosis, this results were not repeated in other study groups who used a cisplatin based regimen as well as in the ongoing French protocol.

The German protocols for testicular (MAHO) and nontesticular (MAKEI) GCTs included cisplatin- and etoposide based chemotherapy regimens. As a result of the excellent event-free survival rates above 80% achieved with the first MAKEI and MAHO protocols, the cumulative chemotherapy was step-wise reduced to 4 resp. 5 cycles without effecting outcome<sup>(16)</sup>. In addition, a risk stratification of chemotherapy according to age, site, histology, stage and completeness of resection has been introduced. According to the current MAKEI 96 protocol, an expectant watch-and-wait strategy is recommended for patients with completely resected low stage tumors. However, patients that relapse during the expectant follow-up require a more intensive regimen with four cycles of three-agent chemotherapy, and as a consequence, therapy is intensified in these patients. In locally advanced and/or metastatic tumors a neoadjuvant approach appears beneficial as it facilitates complete tumor resection and thereby reduces the need for second look surgery<sup>(7,8)</sup>.

#### **SIOP-CNS-GCT 96 protocol on malignant intracranial GCT**

Therapy for malignant intracranial GCT is stratified according to the histologic differentiation (i.e. germinoma vs. secreting GCT) and initial dissemination<sup>(16,17)</sup>. The ongoing SIOP CNS GCT protocol aims to evaluate achieve standard diagnostic procedures which is measurement of markers in serum/CSF, a CSF-cytology and an MRI of head and spine in all patients. Two different therapeutic options in intracranial germinoma with regard to both their therapeutic impact and their specific acute and long-term toxicity. For secreting intracranial tumors and embryonal carcinoma, the effect of a combined treatment with PEI and radiotherapy adapted to dissemination is examined.

In pure intracranial germinoma, which account for 50% of all intracranial GCT and do not secrete significant amounts of HCG/ $\beta$ -HCG, a histologic

verification of the tumor is mandatory. According to the current SIOP CNS GCT 96 protocol patients with germinoma and localized disease can be treated either with craniospinal irradiation with 24 Gy and a tumor boost of 16 Gy or with a multimodal treatment including two cycles of chemotherapy (CarboPEI) followed by a focal irradiation (40 Gy). In metastatic disease craniospinal irradiation and boost to tumor and the metastatic sites is the treatment of choice. Data achieved so far concerning effect of chemo and focal RT reveal that this approach bears a higher risk of ventricular relapses<sup>(18,19)</sup>. Therefore additional ventricular treatment is implemented in new protocols for localized CNS germinoma. It has been demonstrated that a 5 year event free survival of 91% and 5 year overall survival of 94% can be achieved by radiotherapy only<sup>(20)</sup>, whereas due to the higher risk of ventricular failures the 5 year event free survival of patients treated with a combined treatment is about 85 % and the five year overall survival is 92 %. As another important risk factor incomplete staging in germinoma especially focussing on marker evaluation in serum /CSF was detected. More than 50% of the relapsing patients show secretion of markers which had not been measured at initial diagnosis of a germinoma. The secreting intracranial GCT (YST, CHC, EC) show an inferior prognosis compared to germinoma. In these patients 4 cycles of cisplatin based chemotherapy (PEI, **Table 4**) are applied, followed by a delayed tumor resection and radiotherapy. The radiotherapy is stratified according to the initial staging. Non-metastatic tumors receive focal irradiation (54 Gy), whereas patients with intracranial or spinal metastases or tumor cells in the CSF receive a craniospinal irradiation (30 Gy plus 24 Gy tumor boost). The summary of several cooperative protocols and the preliminary data of the SIOP CNS GCT 96 protocol suggest that a long term remission can be obtained in about two thirds of patients. In the SIOP CNS GCT 96 protocol as clinical risk factors AFP > 1000 ng/ml at diagnosis and residual disease after the end of treatment were defined and will be used for definition of risk groups in the forthcoming SIOP CNS GCT II protocol. Additionally in germinoma and non-germinoma; strategies that utilized chemotherapy and excluded radiotherapy have resulted in only insufficient tumor control<sup>(21)</sup>.

**Table 3 : Standard chemotherapy regimen in paediatric GCT**

<b>PEI (MAKEI 96, SIOP CNS GCT 96, MAHO 98, SFOP)</b>			
Cisplatinum <sup>1</sup>	20 mg/m <sup>2</sup>	over 1 h	Day 1,2,3,4,5
Etoposide	100 mg/m <sup>2</sup>	over 3 h	day 1,2,3
Ifosfamide <sup>2</sup>	1500 mg/m <sup>2</sup>	over 20 h	Day 1,2,3,4,5
2 to 4 cycles			
<b>PVB (MAHO 98)</b>			
Cisplatinum <sup>1</sup>	20 mg/m <sup>2</sup>	over 1h	day 4,5,6,7,8
Vinblastin	3 mg/m <sup>2</sup> or 0.15 mg/kg	i.v. bolus	day 1,2
Bleomycin <sup>3</sup>	15 mg/m <sup>2</sup>	over 24 h	day 1,2,3
3 cycles			
<b>BEP (MAHO 98)</b>			
Bleomycin <sup>3</sup>	15 mg/m <sup>2</sup>	over 24 h	day 1,2,3
Etoposide	80 mg/m <sup>2</sup>	over 3 h	day 1,2,3
Cisplatinum <sup>1</sup>	20 mg/m <sup>2</sup>	over 1 h	day 4,5,6,7,8
3 cycles			
<b>BEP (US-Childrens Oncology Group)</b>			
Bleomycin	15 mg/m <sup>2</sup>	over 24 h	day 1
Etoposide	100 mg/m <sup>2</sup>	over 3 h	day 1,2,3,4,5
Cisplatinum <sup>1</sup>	20 mg/m <sup>2</sup>	over 1 h	day 1,2,3,4,5
4 cycles			
<b>High-dose BEP (US-Childrens Oncology Group)</b>			
Bleomycin	15 mg/m <sup>2</sup>	over 24 h	day 1
Etoposide	100 mg/m <sup>2</sup>	over 3 h	day 1,2,3,4,5
Cisplatinum <sup>1</sup>	40 mg/m <sup>2</sup>	over 1 h	day 1,2,3,4,5
4 cycles			
<b>JEB (UKCCSG GCII)</b>			
Carboplatinum	600 mg/m <sup>2</sup>	over 1 h	day 2
Etoposide	120 mg/m <sup>2</sup>	over 1 h	day 1,2,3
Bleomycin <sup>3</sup>	15 mg/m <sup>2</sup>	over 15 min	day 3
5 cycles, or 2 cycles after complete remission			
<b>CarboPEI (SIOP CNS GCT 96)</b>			
Carboplatinum	600 mg/m <sup>2</sup>	over 1 h	day 1
Etoposide	100 mg/m <sup>2</sup>	over 3 h	day 1,2,3,22,23,24
Ifosfamide <sup>2</sup>	1800 mg/m <sup>2</sup>	over 3 h	day 22,23,24,25,26
2 cycles			

<sup>1</sup> plus mannitol forced diuresis, <sup>2</sup> plus Mesna uroprotection, <sup>3</sup> omitted in children < 1 year, 7.5 mg/m<sup>2</sup> in children < 2 years

### Follow-up

A complete clinical remission is defined as normalization of the tumor-markers within the age-related normal range and the absence of suspicious residual structures, even in patients with normalized tumor markers, as these structures may represent remaining mature teratoma. Most relapses occur within the first two years after diagnosis. However, in some patients late recurrences up to five years after diagnosis of malignant ovarian GCT or intracranial germinoma have been observed. Therefore, the initial follow-up examinations after completion of chemotherapy have to be performed in short intervals, (i.e. weekly) controls of the tumor markers AFP and  $\beta$ -HCG early during follow-up. In watch-and-wait patients, the decline of the AFP values is evaluated with regard to its serum half-life of approximately 6 to 7 days.

In addition, the follow-up examinations include repeated imaging of the primary site of tumor. In case of residual structures after chemotherapy of extracranial GCT, resection of these residues is indicated, since mature teratoma may have remained and bear the risk of tumor progression.

In intracranial tumors, endocrinologic tests at diagnosis and during follow-up are mandatory. Also a distinct neurological and if possible neuropsychological evaluation should be obtained. In children treated with cisplatinum-containing polychemotherapy (esp. plus ifosfamide), the renal function has to be monitored carefully for tubular nephropathy and at diagnosis and before every course of platin based treatment audiometry should be performed.

### Relapse treatment

In patients with recurrent or refractory tumors who had previously been treated with a non-platinum or carboplatinum therapy, cisplatinum-based regimens (preferably PEI) have been successfully applied. Therefore, cisplatinum containing regimen in patients with relapsed tumors are preferred, if the organ toxicities related to the previous treatment allow further cisplatinum therapy. Patients suffering from severe cisplatinum-related toxicity may be treated with a combination of carboplatinum and high dose etoposide (at 400-600 mg/m<sup>2</sup> on 3

days). Otherwise, there is no international consensus on strategies for treatment or recurrent GCTs. More than 90% of relapses occur at the primary site of the tumor. Therefore, relapse chemotherapy has to be accompanied by an intensive local therapy, preferably complete resection of the recurrent tumor after tumor-reduction by preoperative chemotherapy. It could be demonstrated that patients with local recurrences and poor response to conventional chemotherapy may profit from locoregional hyperthermia combined with platinum-based chemotherapy. Lastly, high dose local irradiation at doses above 45 Gy has shown some beneficial effect after incomplete resection of the tumor recurrence <sup>(22)</sup>.

For malignant CNS GCT, especially of non-germinomatous histology which failed after first line treatment the chance to achieve a second remission is small. Reports from the French SFOP series and observation within SIOP CNS GCT 96 are that although tumors respond very well again to chemotherapy only in cases with complete biological, removal of any residual tumor and successful applied high dose treatment with additional irradiation a second remission could be achieved.

### Future perspectives

A multimodal approach that utilizes cisplatinum/etoposide chemotherapy as well as tumor resection is highly effective for the treatment of paediatric GCTs. In the light of the high cure rates achieved by current protocols, research must now focus on new aims. Patients should be identified that are only at a low risk of relapse, and in whom adjuvant chemotherapy can either be withheld or significantly reduced, thus allowing to minimize the impact on short- and long-term quality of life, and treatment toxicity. In this context, molecular genetic studies might also reveal some important information that may be utilized for risk stratification. For this rare disease especially for unfavourable sites like CNS tumors international cooperation is vital. Another today's demand is the focus on rehabilitation, reintegration and Quality of Life of the cured patients to determine their quality of survival and if impaired to consider these results for future treatment planning.

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# Diagnostic And Treatment Principles For Low Grade Glioma Of Childhood And Adolescence

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## Introduction

30 – 40 % of paediatric primary brain tumors are low grade glioma. Their annual incidence is calculated to be 10 – 12 per 1.000 000 children under the age of 15 years in western countries. These tumors occur at all ages with a mean age at diagnosis or operation between 6 and 8 years. There is a slight male preponderance (1,1 – 1,2 to 1), although some diagnoses like the DIGG/DIA show a more marked male preponderance.

There is a striking association of specific variants of low grade glioma and heritable diseases which in part may serve as a model for cancer development. 15 – 20 % of patients with neurofibromatosis type 1 develop optic pathway glioma. The occurrence of optic pathway glioma disposes to the development of further brain tumors. Whereas optic pathway gliomas are mostly pilocytic, older age and location outside the visual system predisposes to other histologies and higher malignancy.

The distribution of low grade glioma within the CNS differs according to various published series. In young children tumors of the supratentorial midline dominate, spinal tumors occur in less than 5 %.

## Histology

The clinically used term of low grade glioma confers to tumors of glial origin, usually astrocytic but oligodendrocytic as well. Their histological grade corresponds to grade I and II according to the revised system of the WHO from the year 2000 (Table 1). For clinical purpose some of the mixed glianeuronal tumors are included as well, if their glial component appears most relevant for biologic behaviour. Almost 2/3 of low grade glioma are pilocytic astrocytoma grade I, characterized by Rosenthal fibres and an often biphasic pattern with solid, Rosenthal fibre rich and a more loose, microcystic component. Even

vascular proliferation is compatible with the diagnosis of pilocytic astrocytoma, which also explains why this tumor takes up contrast medium intensively. Regressive changes including calcifications, necrosis and lymphocytic infiltration are more rare. Several subgroups of pilocytic astrocytoma have been defined, their prognostic significance is not validated yet, some seem to be correlated with more aggressive clinical behaviour.

The difficulty to obtain a correct histological diagnosis is highlighted by the fact, that up to a quarter of cases have discrepant diagnoses between local and referent pathology even in large international studies. Even among high grade glioma up to 28 % have been reclassified as low grade glioma by central pathologic review.

Up to now no consistent genetic changes have been associated with low grade glioma. Although pilocytic astrocytoma, associated with NF 1, show the NF-mutation on gene 17q 11.2 regularly, sporadic pilocytic astrocytoma do not show NF 1 gene mutations, but develop rather an overexpression of neurofibromin; in some loss of 17q has been found. The comparative genomic hybridisation point towards few variable patterns. In small series only 5 of 41 analyses were abnormal and concern chromosome 9, 19 and 22. P 53 mutations are rare. Gene expression profiles show distinct patterns of pilocytic astrocytoma with and without NF 1 versus astrocytoma grade II.

The pattern of proliferation of low grade glioma cannot well be explained. They mostly show slow growth with local extension. Spontaneous regression has been reported, but is an exception definitively. Long phases of constant size have been described, explained by an increase of spontaneous apoptosis, reduction of vascular supply or a reduced growth kinetic within the tumor. Some tumors show an

aggressive growth pattern where phases of enlargement and stable disease may interchange. In adults a high proliferation rate identified by Ki 67/MIB 1 index, high levels of VEGF and vascular density or a reduction of N-CAM correlate with a higher rate of tumor progression. In small series of children these findings have been inconsistent. Chiasmatic-hypothalamic tumors did not show a correlation of MIB index and progression following chemotherapy.

A small percentage of low grade glioma may disseminate along the cranio-spinal axis, especially in chiasmatic-hypothalamic glioma of young children. Malignant transformation may be initiated by clonal expansion of cells with P 53 gene mutation.

### Diagnosis

The diagnostic procedure follows generally accepted guidelines for all brain tumors (Table 2).

Preoperative neurologic and ophthalmologic examination is complemented by radiological diagnosis. Pilocytic astrocytomas are hypointense in T 1 with variable contrast uptake in solid parts and in the walls of cysts. These tumors are hyperintense in T 2. Due to their relative paucity of cells in comparison to the cortex native CT shows a hypodense tumor. Calcifications can be found in 10 %. Radiology describes different patterns of growth: cystic with one or several lateral nodules, solid with little cysts or predominantly solid tumors.

Diagnosis without biopsy appears justified in chiasmatic-hypothalamic tumors in the presence of neurofibromatosis or in the presence of extensive visual pathway involvement. Native CT has to prove a hypodense tumor. In all other cases biopsy is necessary.

Multiple cerebral tumors, ependymal or leptomeningeal deposits in the cerebral MRI, and the presence of clinical symptoms necessitate extensive neuro-radiologic diagnosis of the spinal canal. The significance of lumbar cytology has not been evaluated systematically.

### Concept of therapy

The concept for the treatment of low grade glioma starts from surgical resection and

histological diagnosis or from radiological diagnosis. Thereafter it has to be decided, whether a patient can be observed or whether non-surgical treatment has to follow.

### Neurosurgical intervention

Neurosurgical intervention has always been the treatment of choice. The result of the primary resection determines the further course of the tumor disease. Its urgency is determined by the clinical symptoms and the tumor location. Due to the distribution of tumors the extent of resection often is limited to avoid unacceptable postoperative morbidity.

The extent of surgical resection is defined by the judgement of the surgeon and the result of the postoperative radio-imaging, with priority for the MRI-finding. In large cohorts only a 1/3 of tumors can be resected completely, a 1/3 receives subtotal or partial resection and a 1/3 is only biopsied or diagnosed clinically.

Survival in low grade glioma is not the critical endpoint. The natural history of tumors following primary surgical intervention or radiological diagnosis shows a high number of tumor progressions within the first years. The size of the residual tumor is only relevant for the time to progression, which is also determined by tumor localisation. Even the quality of complete resection differs between location of either cerebral hemisphere or cerebellum versus supratentorial midline or spinal canal.

### Non-surgical therapy strategy:

To evaluate the role and impact of non-surgical therapy modalities different questions have to be answered:

- When does non-surgical therapy start – what is the indication?
- What type of treatment has to be chosen – how are the patients stratified?
- What is the ultimate goal of treatment – why shall we treat?

### 1. Radiotherapy

Radiotherapy of low grade glioma has been accepted as standard therapy for decades, but it's optimal role has not been defined up to now. Tumors have been irradiated if they could not

be resected or following incomplete resection or relapse. For children no advantage for routine postoperative irradiation could be shown, so it often was applied at the time of progression of a residual or following incomplete resection of relapse. In small series progression rates are significantly higher if patients have been observed following incomplete surgical resection as compared to immediate irradiation. But 5 and 10 year survival rates have been identical.

The irradiation field has to comprise the clinical target volume, defined by preoperative T 2 weighted tumor extension plus a safety zone corresponding to the infiltration zone which is at least 0,5 cm in pilocytic astrocytoma. The planning target volume has to consider the precision of the radiation technique and add a safety margin of 0,5 – 1,0 cm in case of conventional technique and 0,2 – 0,5 cm in case of rigid head fixation. The aim is to spare neighboring healthy brain parenchyma.

The optimal dose for irradiation of low grade glioma in children has not been determined. The choice of dose and fractionation has been influenced by age of the children, tumor location and tumor size with a tendency for lower dose in young children. Retrospective analysis of small series allows to recommend doses between 45 and 54 Gy in fractions of 1,8 Gy for older children.

To adequately evaluate the effect of radiotherapy a couple of aspects have to be considered: Tumor volume response is not directly correlated with tumor control and improvement of symptoms. The percentage of tumors with radiological regression is around 50 %. There is a great variability concerning the response over time, tumor volume response is mostly delayed but continues for years. The demarcation of regressive changes from those in case of progression is sometimes difficult with a maximum size following radiotherapy occurring up to 9 – 12 months.

While radiotherapy allows for tumor volume regression and prolonged PFS its well documented late effects, especially concerning psychointellectual development, in the developing brain, make its use in small children and children with neurofibromatosis non attractive. Age at radiation and the length of follow-up are important to truly estimate the

impact of radiation damage. Visual-spacial capacities are damaged by the tumor and its therapy. In all tested areas performance of children without NF1 is inferior following radiotherapy as compared to chemotherapy alone. Children with neurofibromatosis show significant neurophysiologic impairment even following chemotherapy alone. Additional late effects are endocrinopathies and vasculopathies, especially in NF 1. The incidence of second malignancy has not been evaluated separately for low grade glioma. But the risk for radiotherapy induced second brain tumors is higher in young children.

## 2. Chemotherapy

The indication to test chemotherapy has therefore been the attempt to delay the start of an eventual radiotherapy. The claim of chemotherapy-protocols has not been to replace radiotherapy but to defer its use to a higher age. It has not been evaluated yet, whether radiotherapy can be avoided in some children completely. Side-effects of chemotherapy concerning psychointellectual and endocrine development of children are less relevant.

This strategy has been accepted for small children, especially for those with large tumors, and for children with NF 1 who tend to develop further even more malignant brain tumors. In older children the rationale for the use of primary chemotherapy is less clear cut. But it can be sensible to treat prepubertal children with hypothalamic tumors to avoid impaired growth with chemotherapy.

Almost all chemotherapeutic agents have been tested for low grade glioma. Formal phase II-studies are only available for some of the them. Combination therapy achieves response rates of 75 – 100 %. For these often irregularly shaped tumors determination of response following classic patterns is often impossible. Tumor stabilisation is an adequate response for the chemotherapy strategy and is therefore included if response rates are reported.

Chemotherapy studies focussing upon young children and upon NF 1 patients

- The first CCSG study applied Carboplatin and Vincristin to 78 children with low grade glioma of all CNS localisation. Median age at

diagnosis was 37 months, 19,2 % had NF 1. After a median observation time of 30 months (range 18 – 72 months) 3 year PFS was 68 % with 76 of 78 children surviving. Younger children under 5 years achieved a more favorable PFS of 74 % +/- 7 % after 3 years as compared to older children with a PFS of 39 % +/- 21 %. NF 1 status and response to chemotherapy did not show statistical differences.

- The study of the French Society of Paediatric Oncology comprised 85 children with progressive visual pathway gliomas. The median age at start of therapy was 33 months, the percentage of NF 1 patients was 27 %. After an intensive 16 months chemotherapy with 6 drugs results have been reported following an observation time of 6,5 years (range 1,8 – 11,5 years). 5 years-PFS was 34 % and overall survival 89 %. The 5 year radiotherapy free survival was 61 %. Age under 1 year with a 3 year-PFS of 34 % was a significantly unfavorable factor as well as poor response to the first cycle of chemotherapy. Children without NF 1 fared less well in univariate analysis.
- The small series treated at the Instituto Nazionale Tumori in Mailand comprised 34 children with low grade glioma of all CNS location. Median age at start of treatment was 45 months and 24 % had neurofibromatosis. The 10 – 11 months chemotherapy with Cisplatin and Etoposide was applied at growing intervals. Following a median observation time of 44 months (range 10 – 120 months) resulted in a 3 year PFS of 78 % and an overall survival of 100 %. The log rank test showed age under 1 year to be a prognostic unfavorable factor with a 3 year-PFS of 33 %.
- The first trial of the International Society for Paediatric Oncology recruited 201 patients with low grade glioma of all CNS locations. Median age at diagnosis was 35,6 months and 21,1 % had NF 1. Chemotherapy lasted 53 weeks with single high dose of Carboplatin and Vincristin. 3 year PFS was 57,5 % and 5 year PFS 45,2 %. 3 year overall survival was 89,1 %. Again age under 1 year was prognostically unfavorable, but also the necessity of an initial tumor reductive

intervention. Leptomeningeal dissemination and absence of neurofibromatosis were significant only in the univariate analysis.

Taken all together the analysis of the series demonstrates that chemotherapy is effective, it produces responses and is able to delay the necessity for radiotherapy in a significant proportion of children. Long term PFS has been unsatisfactory however and in some series there is a significant number of early progressions. Up to now potential prognostic factors have not been defined prospectively, but age, NF-status, extent of primary response, start of therapy or/ and histology may be some of these influential factors. One of the difficulties to compare the series has been the lack of uniform criteria for the start of non-surgical therapy, in either radiotherapy or chemotherapy trials. And there has been no uniform definition of clinical or radiological progression.

The definition of clinical or radiological indication to start non-surgical therapy is therefore crucial to the comparability of different trials. It is generally accepted that the presence of a (postoperative) tumor is no indication to therapy by itself at diagnosis. Only severe clinical, neurologic or ophthalmologic findings justify the start of non-surgical therapy, be it following partial resection or neuroradiological diagnosis. Following an observation time ophthalmologic, radiologic or neurologic progression is a sufficient indication if no tumor resection is possible. For each of the various fields clear-cut further definitions have to be defined.

### Study objectives

Overriding aims of all studies of therapy for low grade glioma have to be:

- To provide a comprehensive standardized concept to treat children and young adults with a low grade glioma of all histologies and locations.
- To improve PFS for young children following chemotherapy. The options to achieve this goal are:
- Improve the choice of drugs, but there has not been a chemotherapeutic agent with a specifically favorable effect.
- Improve the intensity of therapy, but it has

not been investigated, whether more therapy improves response and whether an improved response confers a better PFS.

- Optimize the duration of therapy. Longer treatment seems to improve PFS.
- Children that have to receive radiotherapy shall be treated following modern 3-dimensional planning and by the use of modern technique in order to reduce the late effects upon normal surrounding brain.

Radiotherapy should only be applied to older children and total dose and fractionation should be maintained at 54 Gy for the brain and 50,5 Gy for the spinal canal at 1,8 Gy dose per fraction. Younger children should only be irradiated following consultation of national reference radiotherapists. For disseminated tumors a trial of crano-spinal irradiation can be done following progression after primary chemotherapy. However in such phase II-studies the volume of irradiated tumor and metastases has to be limited.

For all low grade glioma studies the feasibility of the strategy has to be examined as well as overall survival, event free and progression free survival following diagnosis. Additionally the effects of tumor and treatments concerning neurology, endocrinology, ophthalmology and health associated factors (quality of life, health status) have to be investigated. A systematic follow-up should especially be instituted for visual function, since data for either chemotherapy or radiotherapy are totally lacking.

Due to the rarity of these tumors large international collaboration should be instituted to achieve significant results within a reasonable space of time.

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