SIOP EDUCATION BOOK 2009

International Society of Paediatric Oncology

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Acknowledgements

The International Society of Paediatric Oncology would like to acknowledge the efforts of the authors. SIOP is immensely grateful to all the authors for not only presenting state of the art lectures and occasional sessions at the meeting but for agreeing to produce a manuscript to assist in ongoing education for participants at the 41st meeting of SIOP in Sao Paulo 2009.

Disclaimer

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

Authors ............................................................................................................................... 8
Preface ............................................................................................................................... 10

Section A – Educational Session:
State of the Art - Soft Tissue Sarcomas

1. The Epidemiology of Soft Tissue Sarcomas
   Karina Ribeiro ........................................................................................................... 16

2. Soft Tissue Sarcomas: Molecular pathogenesis
   Timothy Triche .......................................................................................................... 23

3. Gene expression profiling of soft tissue sarcomas
   Isabela Werneck da Cunha ...................................................................................... 32

4. Soft tissue sarcoma of the extremities
   J.C. Barbi Goncalves ................................................................................................. 35

5. Radiotherapy in Rhabdomyosarcoma
   Tobias Bölling .......................................................................................................... 41

6. Particularities on diagnosis and management of
   non-rhabdomyosarcoma soft tissue sarcomas
   Andrea Ferrari .......................................................................................................... 45

7. Role of Chemotherapy in Non-Rhabdomyosarcoma
   Soft Tissue Sarcoma
   Milena Villarroel ...................................................................................................... 50

8. Surgery of non-rhabdomyosarcoma soft tissue sarcoma
   Samuel Aguiar .......................................................................................................... 57

9. Radiotherapy in the management of Pediatric Non-Rhabo
   Soft Tissue Sarcoma.
   Valerie Bernier ....................................................................................................... 62
Section B – Keynote lectures

1. Pediatric Acute Myeloid Leukemia—Improving Survival One Patient at a Time
   Raul Ribeiro, Ina Radtke, Jeffrey Rubnitz .......................................................... 69

2. Prognostic Factors in Wilms Tumor of the Kidney
   Paul Grundy ........................................................................................................ 79

3. Surgery for Rare Tumors in children: The experience of the Italian TREP Project
   Giovanni Cecchetto ...................................................................................... 88

4. Infections in Children with Acute Myeloid Leukemia: Lessons Learned from International Co-operative Group Trials and Low Income Countries
   Lillian Sung .................................................................................................. 95

5. Building evidence based practice in paediatric oncology
   N. Kline ........................................................................................................ 99

6. Nephrogenic Rests and Nephroblastomatosis
   Elizabeth Perlman ...................................................................................... 101

7. Epigenetic Maintenance of Stemness in Pediatric Malignancy
   Stefan Burdach & Günther HS Richter ............................................................ 106

8. Rationale for tumour prevention of HPV vaccination in adolescents
   Luisa Villa .................................................................................................. 113

9. Oncogenic Pathways in Embryonal Tumors
   Angelika Eggert ...................................................................................... 117
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Preface: SIOP EDUCATION BOOK 2009

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Gabriele Calaminus
Chair Elect, Scientific Committee, SIOP
Preface: SIOP Education Book 2008

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

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

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**Preface: SIOP EDUCATION BOOK 2007**

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

Tim Eden  
President, SIOP

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Chair, Scientific Committee, SIOP

Bharat Agarwal  
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Gabriele Calaminus  
Chair Elect, Scientific Committee, SIOP
Preface: SIOP EDUCATION BOOK 2006

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

Enjoy the book and the talks.

Tim Eden
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Pierre Wacker
Local Organizing Committee
38th Congress of SIOP
Preface: SIOP EDUCATION BOOK 2005

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

Tim Eden
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Paul Rogers
Local Organizing Committee
37th Congress of SIOP
The Epidemiology of Soft Tissue Sarcomas

Abstract
Soft tissue sarcomas comprise about 4-8% of all cancers among children (0-14 years) in Europe, Asia, and Americas. Pediatric STS are distinct from adult STS regarding incidence rates, frequency of histological types, biological behavior and prognosis. Rhabdomyosarcomas (RMS) represent more than half of all pediatric STS. Very little is known about risk factors for STS and definitive evidences exist only for a few genetic syndromes and congenital abnormalities, although these associations can explain only a small proportion of the cases.

Introduction
Pediatric soft tissue sarcomas (STS) include neoplasms of miscellaneous histological types arising from mesenchymal cells at any anatomic body site. They are distinct from adult STS regarding incidence rates, frequency of histological types, biological behavior and prognosis. Among children, these neoplasms are classified in two broad groups: rhabdomyosarcomas (RMS), representing more than half of all pediatric STS, and non-rhabdomyosarcoma soft tissue sarcomas (NRSTS), which includes fibrosarcoma, synovial sarcoma, leiomyosarcoma, dermatofibrosarcoma protuberans, malignant fibrous histiocytoma, malignant peripheral nerve sheath tumor (MPNST), and Kaposi sarcoma, among others (Table 1).

Table 1: Classification of STS, according to histological type and the corresponding group on the International Classification of Childhood Cancer – 3rd edition (ICCC-3)61

<table>
<thead>
<tr>
<th>Group</th>
<th>Histological Type</th>
<th>ICCC group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhabdomyosarcomas</td>
<td>Pleomorphic RMS</td>
<td>IXa</td>
</tr>
<tr>
<td></td>
<td>Embryonal RMS</td>
<td>IXa</td>
</tr>
<tr>
<td></td>
<td>Alveolar RMS</td>
<td>IXa</td>
</tr>
<tr>
<td>Non-rhabdomyosarcoma soft tissue sarcomas</td>
<td>Fibrosarcoma</td>
<td>IXb</td>
</tr>
<tr>
<td></td>
<td>Neurofibrosarcoma</td>
<td>IXb</td>
</tr>
<tr>
<td></td>
<td>Infantile fibrosarcoma</td>
<td>IXb</td>
</tr>
<tr>
<td></td>
<td>Malignant fibrous histiocytoma</td>
<td>IXb</td>
</tr>
<tr>
<td></td>
<td>Dermatofibrosarcoma protuberans</td>
<td>IXc</td>
</tr>
<tr>
<td></td>
<td>Kaposi's sarcoma</td>
<td>IXd</td>
</tr>
<tr>
<td></td>
<td>Hemangioendothelioma</td>
<td>IXd</td>
</tr>
<tr>
<td></td>
<td>Leiomyosarcoma</td>
<td>IXd</td>
</tr>
<tr>
<td></td>
<td>Liposarcoma</td>
<td>IXd</td>
</tr>
<tr>
<td></td>
<td>Angiosarcoma</td>
<td>IXd</td>
</tr>
<tr>
<td></td>
<td>Synovial sarcoma</td>
<td>IXd</td>
</tr>
<tr>
<td></td>
<td>Epithelioid sarcoma</td>
<td>IXe</td>
</tr>
</tbody>
</table>
The majority of RMS are located in the head and neck region (35-37%), followed by genitourinary sites (21-25%), and extremities (13-20%). In Intergroup Rhabdomyosarcoma Study-II (IRS-II), 34% of all tumors were located in the head and neck: 18% were parameningeal (middle ear, nasal cavity, paranasal sinuses, nasopharynx, mastoid, temporal region, pterygopalatine and infratemporal fossa), 8% were located in the orbit and 8% in other sites of head and neck (including parotid gland, larynx, oropharynx, oral cavity, scalp, thyroid and parathyroid glands, and neck). Embryonal RMS is the predominant histological subtype (about 60% of all pediatric RMS), while alveolar subtype corresponds to about 20-25%, being more frequent in older children and adolescents (10-19 years, more than 30% of all RMS).

Among pediatric NRSTS, the most common histological types are dermatofibrosarcoma protuberans, synovial sarcoma, MPNST’s, malignant fibrous histiocytoma, and fibrosarcoma, with a predominance of tumors located in the limbs.

Substantial improvements in understanding the tumor biology with consequent better treatment modalities through the establishment of cooperative groups have resulted in survival rates rising from 30-40% in 1970’s to 60-70% currently.

**Descriptive Epidemiology**

STS comprise about 4-8% of all cancers among children (0-14 years) in Europe, Asia, and Americas. However, in some African countries, STS are rather more frequent (15-40% of all childhood neoplasms) and this can be explained by the HIV epidemic and the high incidence of Kaposi sarcoma.

Age-adjusted incidence in Europe is 9.1/million, with a particular high incidence of RMS among infants living in the Nordic countries (Denmark, Finland, Norway, and Iceland, 10.4/million). Data from the National Program of Cancer Registries (NPCR) show an overall STS age-adjusted incidence of 10.4/million in United States for children less than 15 years of age, with a considerable variation across different states, ranging from 7.2/million in Nebraska to 16.0/million in Rhode Island. Similar rates are observed in São Paulo, Brazil, for children less than 19 years of age (Males: 13.8/million; Females: 11/million). In Japan, according to data from the Hokkaido Children’s Cancer Registry, age-adjusted incidence rate (0-14 years) of STS over the period of 1975-1999 was 4/million.

In most of the populations, boys present higher incidence rates than girls. According to data from the Automated Childhood Cancer Information System (ACCIS), an excess of RMS in boys has been observed in Europe (male:female ratio = 1.4). In the United States, for all STS rates among males also tend to be higher than for females, although difference is smaller (male:female ratio=1.1).

Age-specific incidence rates for RMS peaks at 1-4 years of age, decreasing subsequently with a slightly increase later at age group 15-19 years, for both males and females (Figure 1A). Age distribution for NRSTS is quite distinct of RMS, with a U-shape, with incidence peaks among infants under 12 months and adolescents between 15 and 19 years of age (Figure 1B).
There are few reports on ethnic differences in the incidence of STS. In the Great Britain, it has been described that children of South Asian origin (Indian, Pakistani or Bangladeshi) have a lower incidence of RMS compared to non-South Asian children (standardized rate ratio = 0.37, 95% CI 0.15-0.95). In the United States, Hispanic children present incidence rate similar to that observed for non-Hispanic white children, both for STS overall and RMS, individually. However, rates are slightly higher among black compared to white children.

Time trends
In Europe, the incidence of STS increased 1.8% per year over the period 1978-1997, due to significant increases observed for embryonal RMS (Average Annual Percent Change=1.7) and other specified STS (AAPC=2.8). A smaller increasing trend was observed in the United States, over the period 1973-2005 (AAPC=0.7, p<0.05), but his was exclusively due to an increase in incidence of other specified STS (AAPC=1.6).

Also, in Japan, an increasing trend has been observed for the whole group of STS, as well as for RMS, with a reported age-adjusted rate of 1.9/million over the period 1971-1980 and 4.3/million for the period 1981-1988.

Survival
In Europe, according to the last report by EUROCAR-4, five-year survival for STS was 67.5%, Eastern Europe presented the lowest rate (47.4%), while the highest was observed in Southern Europe. For RMS specifically, the observed 5-year survival rate was 69.1% for Europe as a whole, and a very high rate was noted in Northern Europe (78.4%). No significant survival changes over time were observed when comparing rates registered for children and adolescents diagnosed with STS in 2000-2002 versus 1995-1999. Previous results from EUROCAR-3 had shown a significant increasing trend in survival rates for RMS over the period 1983-1994 (1983-1985=59.0%; 1986-1988: 61.2%; 1989-1991: 65.1%; 1992-1994: 67.4%, p=0.013). In the United States, data from SEER point a significant change in 10-year survival rates for children with RMS, with rates increasing from 53.8% (1975-1984) to 64.5% (1985-1994) and particular improvements were observed for males (+13%), whites (10%), and children ages 1 to 4 years (+25%). On the other hand, stable rates were observed for NRSTS (73.7% and 73.0%, respectively).

Second primary tumors (SPT)
An analysis of data from SEER, including 1,759 survivors of pediatric STS diagnosed over the period 1973-2000, has shown a six-fold increased risk of SPT for children with STS. The risk was increased for all histological subtypes of STS and most common subsequent malignancies include acute non-lymphocytic leukemia (ANLL), melanoma, bone and soft tissue sarcomas, breast and oral cavity cancers. In addition, it has been demonstrated that the combination of radiotherapy and chemotherapy is associated with a significantly higher risk of SPT and the observed excess of ANLL has been attributed to therapy with alkylating agents and DNA topoisomerase II inhibitors. Moreover, data from the Childhood Cancer Survivor Study has revealed that childhood cancer survivors overall have an increased risk of developing a secondary sarcoma (SIR=9.0, 95%CI 7.4-10.9) with especially higher risks for those survivors of other STS, bone sarcomas, kidney and CNS tumors and Hodgkin lymphoma. Radiation therapy and chemotherapy regimens including high doses of anthracyclines (>300 mg/m², RR=2.3, 95%CI 1.2-4.3) or alkylating agents (alkylator dose, upper tercile, RR=2.2, 95%CI 1.1-4.6) were also associated to increased risk of secondary STS, while family history of cancer was only marginally significant in the multivariate analysis (RR=1.4, 95%CI 0.9-2.1).

Risk Factors
Genetic Factors
Genetic syndromes and congenital abnormalities are the only well-established risk factors for STS, although they account only for a small proportion of the cases, since the vast majority is sporadic. STS are mainly associated with familial cancer syndromes such as Li-Fraumeni Syndrome (LFS) and Neurofibromatosis type I (NF1), but also less frequently with Beckwith-Wiedemann Syndrome, Hereditary Retinoblastoma, Neviod Basal Cell Carcinoma Syndrome, and Costello Syndrome.

LFS was first described based on a review of
medical charts and death certificates of 648 children with RMS, which has resulted in the identification of five families in which siblings or cousins had a childhood sarcoma. LFS is an autosomal dominant disorder predisposing for sarcomas (bone and soft tissue), breast cancer, brain tumor, adrenocortical carcinoma and leukemia. Germline mutations of the p53 gene are the main cause of this syndrome, being detected in approximately 70% of the affected families.

Patients affected by NF1 are at higher risk of developing STS than the general population, particularly MPNST and RMS. Most patients present an urinary RMS. NF1 is caused by a mutation in the neurofibromin gene, located on chromosome 17q11.2 and it is characterized by café-au-lait spots, learning disabilities, and fibromatosus tumors. The prevalence of NF1 among children with RMS ranges from 1 to 6%, while in the general population is approximately 0.03-0.4% at birth. Cross-sectional studies have shown that 1 to 2% of patients with NF1 develop MPNST.

RMS is also associated with congenital abnormalities. A cohort-linkage study based on the follow-up of 90,400 children has identified that children with birth defects have a higher risk of developing STS (RR=1.9; 95%CI 1.0-3.5). In an autopsy study, congenital anomalies were identified in 37 of 115 (32%) children and adolescents with RMS, without differences regarding sex, age, site, or histology. The distribution of the anomalies by system included central nervous (9), genitourinary (10), gastrointestinal (13), and cardiovascular systems (4). Children with RMS had a significant higher prevalence of major malformations (6.0%) than controls (2.6%) (OR=2.4, 95%CI 0.9-6.2). In addition, authors have observed in this study a concordance between the anatomic site of the RMS and the major congenital abnormality for 40% of the cases, suggesting that the same genetic change or intrauterine exposure could disturb the normal embryo development originating structural defects, and thus predisposing the child to embryonal cancer.

RMS subtypes have distinct molecular and genetic profiles. Alveolar RMS is associated with translocations involving two PAX genes (thought to be important for muscle development during embryogenesis) at chromosomes 2q35 (PAX3) and 1p36 (PAX7) with the FKHR gene at chromosome 13q14. The t(2;13)(q35;q14) (found in 55% of the tumors), and t(1;13)(p36;q14) (found in 22% of the tumors) translocations result in the PAX3-FKHR and PAX7-FKHR fusion genes, respectively. However, 25% of the tumors, in spite of displaying the classic alveolar histology, lack either translocation, suggesting that fusion status assessment might be considered in the future for prognostic stratification for children with alveolar RMS.

In contrast to these specific translocations, genetic changes in embryonal RMS include frequent loss of heterozygosity (LOH) at chromosome 11p15.5, a locus enclosing many growth-related imprinted genes involved on tumorigenesis such as H19, IGF2, and p57. LOH studies have also indicated the existence of presumed tumor suppressor genes located at long arms of chromosomes 11 and 16. Other genetic changes found in RMS include amplification of MYCN, MDM2, and CDK4, mutations in RAS genes, as well as gains on chromosomes 2, 8, 12, and 13.

Chromosomal abnormalities are also commonly found for NRSTS. Congenital fibrosarcoma, dermatofibrosarcoma protubersans, and synovial sarcoma present chromosomal translocations t(12;15)(p13;q25), t(17;22)(q21;q13), and t(X;18)(p11;q11), respectively, with the resulting fusion transcripts is important for muscle development during embryogenesis) at chromosomes 2q35 (PAX3) and 1p36 (PAX7) with the FKHR gene at chromosome 13q14. The t(2;13)(q35;q14) (found in 55% of the tumors), and t(1;13)(p36;q14) (found in 22% of the tumors) translocations result in the PAX3-FKHR and PAX7-FKHR fusion genes, respectively. However, 25% of the tumors, in spite of displaying the classic alveolar histology, lack either translocation, suggesting that fusion status assessment might be considered in the future for prognostic stratification for children with alveolar RMS.

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Radiation

A case-control study of 319 RMS cases and 319 matched controls described an association between prenatal diagnostic X-ray exposure and risk of RMS in children (OR=1.9, 95%CI 1.1-3.4 for any X-ray examination). The highest increase in risk was observed for exposures occurring during the first trimester (OR=5.7; 95% CI, 1.2-27.8) and was also increased for the third one (OR=2.0; 95% CI, 0.9-4.6), whereas second trimester exposure was not associated with...
increased risk. Increased risk was significantly associated with “other” X-ray exposures (OR=2.9; 95% CI, 1.1-7.7), mainly composed of dental X-rays. The association was strongest between embryonal RMS and first trimester exposure (OR=10.5; 95% CI, 1.5-458.4)\textsuperscript{52}.

**Infections and Immunizations**

Grufferman et al reported that children with RMS received fewer routine childhood immunizations than controls, describing a particular association with smallpox vaccination (RR=0.2, 95%CI 0.1-0.6)\textsuperscript{53}. Data from the same study have shown significant positive associations with child’s history of whooping cough (OR=4.1, 95%CI 1.1-15.0) and impetigo (RR=4.3, 95%CI 1.0-18.0), as well as a borderline association with measles (RR=2.0, 95%CI 0.9-4.6)\textsuperscript{53}. However, further studies did not confirm these findings.

Maternal use of antibiotics within one year preceding or during the index child pregnancy was associated with a higher risk of RMS (RR=2.7, 95%CI 1.1-6.5). Another case-control study confirmed this finding, showing an increased risk of STS has been described for children whose mothers had used antibiotics during the neonatal period (OR=6.8, 95%CI 1.1-71.2), which was mainly drive by the specific association with RMS (OR=5.9, 95%CI 0.9-64.9)\textsuperscript{54}.

HIV infection is associated with leiomyosarcoma, which is the second leading cancer among HIV+ children, presenting mostly in the gastrointestinal tract, although unusual locations such as spleen, lungs, and adrenal glands have also been reported\textsuperscript{55}. In these cases, Epstein-Barr virus (EBV) appears to be an important etiological co-factor, being detected in tumor tissue by in situ hybridization and quantitative PCR\textsuperscript{56, 57}. Leiomyosarcomas are also reported in immunocompromised children following liver and kidney transplantation\textsuperscript{56}.

**Lifestyle**

**Paternal and maternal smoking**

Paternal smoking was associated to an increased risk of RMS (RR=3.9, 95%CI 1.5-9.6) in one case-control study\textsuperscript{53}, but this finding was not replicated in subsequent studies\textsuperscript{54, 59, 60}. Maternal smoking did not confer an increased risk of STS in any of the published case-control studies\textsuperscript{53, 54, 59, 60}.

**Maternal and Paternal Recreational Drug Use**

A case-control study carried out in United States, including 322 RMS cases (0-20 years) and 322 controls, have identified that both maternal (OR=3.0, 95%CI 1.4-6.5) and paternal (OR=2.0, 95%CI 1.4-6.7) use of marijuana during the year preceding their child’s birth was associated with an increased risk of child developing RMS. Maternal cocaine use was associated with a 5.1-fold increased risk (95%CI 1.0-25.0), while risk related to paternal consumption was lower (OR=2.1, 95%CI 0.9-4.9). Maternal steroids use also yielded a higher risk of RMS in the offspring (OR=3.7, 95%CI 1.3-10.4)\textsuperscript{59}.

**Socioeconomic Status (SES)**

Low socioeconomic status has been associated with increased risk of STS. A first study has shown that both parents of children with RMS had lower education than controls parents, and also that case parents’ had less prestigious occupations and lower family income compared to controls\textsuperscript{53}. Subsequently, in a national case-control study to investigate risk factors for RMS, Grufferman et al reported that case families (32% with total annual family-income less than 20,000 USD) had lower incomes than control families (24% with total annual family-income less than 20,000 USD) (OR=1.52, 95% CI 1.06-2.19)\textsuperscript{59}.

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Soft Tissue Sarcomas: Molecular pathogenesis

Timothy J. Triche

Abstract
The two major mesenchymally derived malignancies in children and adolescents are leukemia and sarcoma. Both groups have a high proportion of subtypes with specific gene translocations, first identified by cytogenetics and later confirmed by PCR and DNA sequencing. Among soft tissue sarcomas, at least a dozen such translocations have been documented, notably the PAX-FOXO1A translocation in alveolar rhabdomyosarcoma, the EWS-ets (most commonly FLI1 or ERG) in Ewing’s sarcoma of bone or soft tissue, and the SYT-SSX1 or 2 in synoviosarcoma. Others have been described in desmoplastic small round cell tumor, liposarcoma, chondrosarcoma, clear cell sarcoma, alveolar soft part sarcoma, inflammatory myofibroblastic tumor, dermatofibrosarcoma protuberans, and clear cell sarcoma. While clearly pathogenic in these sarcomas, they alone are insufficient to cause malignancy. A multitude of additional genomic abnormalities are necessary. Here we review the more common chimeric genes, their known downstream gene targets, and compare translocation positive sarcomas with those that lack such obvious, recurring gene translocations. Gross abnormalities in DNA copy number (chromosomal loss or gain, regional amplification, deletions), loss of heterozygosity, and uniparental isodisomy are common in this group (but may be found to some extent in the translocation positive cases as well). The specific genetic defects that normally lead to sarcomas in humans are not yet known. Newly developed whole-genome sequencing methods may soon allow identification of every recurring genetic defect in these tumors and lead to an understanding of critical ‘driver’ defects and ‘passenger’ anomalies that may create the permissive environment on which the ‘driver’ defects operate. Ultimately, the challenge will be to distinguish pathogenic genomic alterations from ‘passenger’ genomic alterations. These alterations may include changes in DNA sequence, copy number, allelic loss, translocations, methylation, and other epigenetic changes that result in altered transcriptional activity, evidenced by alternate RNA transcripts, with alternate exon usage, intron inclusion, altered intron-exon boundaries, alternate transcription start and start sites, themselves resulting in altered protein gene products and, perhaps more importantly altered regulatory networks. Experience with genome-wide association studies (GWAS) and in vitro studies of chimeric gene transfectants suggest that no one alteration will be responsible for the altered biologic properties we recognize as cancer. Rather, a set of alterations, perhaps as few as ten (as suggested by recent studies of AML) may be minimally sufficient. Conversely, some tumors clearly possess hundreds of genomic anomalies that may contribute to the malignant phenotype. These observations in aggregate suggest the pathogenesis of sarcomas in particular, and cancer in general, is a multi-defect process, the nature of which to date is poorly understood. Future work will need to focus on total genomic characterization of cancers in order to dissect out the critical factors leading to and maintaining the malignant phenotype.

Common Genomic Alterations in Sarcomas:
Dramatic chromosomal alterations have been noted in leukemia and sarcomas from the earliest days of tumor cytogenetic analysis. The most common cytogenetic anomalies and their corresponding molecular defects are summarized in Table 1.
**Table 1: Cytogenetic anomalies & corresponding molecular defects**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Cytogenetics</th>
<th>Molecular Lesion</th>
<th>Molecular Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewing family tumors (EFTs)</td>
<td>t(11;22)(q24;q12)</td>
<td>EWS-FLI1</td>
<td>EWS-FLI1 FISH (2 color)</td>
</tr>
<tr>
<td></td>
<td>t(21;22)(q22;q12)</td>
<td>EWS-ERG</td>
<td>EWS FISH (breakapart)</td>
</tr>
<tr>
<td></td>
<td>t(7;22)(p22;q12)</td>
<td>EWS-ETV1</td>
<td>EWS FISH (breakapart)</td>
</tr>
<tr>
<td></td>
<td>other 22q12</td>
<td>Other EWS fusions</td>
<td>EWS FISH (breakapart)</td>
</tr>
<tr>
<td>Alveolar rhabdomyosarcoma (RMS)</td>
<td>t(2;13)(q35;q14)</td>
<td>PAX3-FKHR</td>
<td>FKHR FISH</td>
</tr>
<tr>
<td></td>
<td>t(1;13)(p36;q14)</td>
<td>PAX7-FKHR</td>
<td>FKHR FISH</td>
</tr>
<tr>
<td>DSRCT</td>
<td>t(11;22)(p13;q12)</td>
<td>EWS-W T1</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>CCSSSP</td>
<td>t(12;22)(q13;q12)</td>
<td>EWS-ATF1</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>ALCL</td>
<td>t(2;5)(p23;q35)</td>
<td>NPM-ALK</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>Synovial Sarcoma</td>
<td>t(X;18)(p11.2;q11.2)</td>
<td>SYT-SSX1</td>
<td>PCR, FISH</td>
</tr>
<tr>
<td>CFS and CMN</td>
<td>t(12;15)(p13;q25)</td>
<td>ETV6-NTRK3</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>ASPS</td>
<td>t(X;17)(p11;p25)</td>
<td>ASPL-TFE3</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>IMT</td>
<td>t(1;2)(q22-23;p23)</td>
<td>TPM3-ALK</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td></td>
<td>t(2;19)(p23;p13.1)</td>
<td>TPM4-ALK</td>
<td>PCR and sequence</td>
</tr>
</tbody>
</table>

Virtually all of these recurring, tumor-specific translocations result in the creation of a biologically functional chimeric gene with DNA transcriptional activity. They are readily detected by both PCR and FISH, and because occasional novel translocation partners are encountered, both assays are useful in establishing a diagnosis. A typical result for synoviosarcoma is shown in **Figure 1**:

**Figure 1:** Synoviosarcoma cytogenetics (left panel) and corresponding molecular defect, creation of the SYT-SSX 1 or 2 chimeric gene (right panel). Note that SSX1 and 2 are distinguished by specific primers. Figure kindly provided by Dr. Deborah Schofield.

**Chimeric Gene Function in Sarcomageneis**

It is intuitively obvious that the nearly 1:1 association of chimeric gene with a given sarcoma suggests a critical function of the chimeric gene in the genesis of the sarcoma. However, numerous studies have documented that introduction of the chimeric gene does not result in a sarcoma. On the contrary, in an inappropriate background, the transduced cells die and no sarcoma results. On the other hand, successful introduction of the chimeric gene in a permissive background appears to 'hijack' the tumor.
phenotype to one more commonly associated with that chimeric gene. This has been shown by several groups in the case of the Ewing’s EWS-FLI1 chimieric gene, for example; when introduced into a fibroblastic, myoblastic, or neural background, a tumor that resembles Ewing’s results. Thus, the more reasonable function of at least some tumor chimeric genes appears to be a differentiation program that recapitulates the native tumor, even in a foreign background (Figure 2). Further, the lack of oncogenesis in a normal or non-permissive background indicates these chimeric genes are more related to phenotype than malignant transformation. It is thus imperative to understand the multiple genetic defects that ultimately result in a given sarcoma type.

**Figure 2:** EWS-FLI1 transfectant into a rhabdomyosarcoma background. Expression of the Ewing’s chimeric gene creates a Ewing-like tumor phenotype and abrogation of the native myogenic phenotype.

**Chimeric Gene Targets**

A number of studies have now identified typical ‘downstream’ gene targets of the chimeric genes commonly found in many sarcomas. Surprisingly, the spectrum is diverse and the functional consequences are not intuitively obvious. Typical classes include cell cycle, signaling, and pathways. These are illustrated in Figure 3 for the EWS-FLI1 gene in Ewing sarcoma. However, other authors have found many additional gene targets, and their functional inter-relationships, if any, remain unclear.

**Figure 3:** Ewing target genes and potential pathway relationships.
Additional Genomic Abnormalities

If chimeric genes alone do not explain sarcomagenesis, what does? This is a difficult question to answer. Both translocation positive and negative sarcomas display a host of genomic anomalies, generally more in the sarcomas that lack a translocation. One interpretation of this observation is that chimeric genes contribute one element of the many that are necessary for malignant transformation. The multiple other genetic defects are more difficult to categorize, as they are multiple, non-repetitive, and not associated with a specific tumor type in most cases. However, they can be grouped broadly by frequency within a given sarcoma type. This is the case with rhabdomyosarcoma, where the alveolar subtype in virtually all cases possesses a typical PAX3 or PAX7 fusion with FOXO1A (aka FKHR), while the embryonal subtype has no known recurring gene translocation. However, a total genome scan using SNP arrays capable of detecting DNA copy number (CNV) and allelic loss (LOH) clearly shows a strong association between the embryonal subtype and chromosome 11 LOH, as well as a number of other chromosomal segment loss or gains. This is illustrated in Figure 4. This observation strongly suggests that pathogenic alterations in DNA CNV and sequence variation (SV), either acquired (mutations) or inherited (polymorphisms), are of major importance in these sarcomas.

Figure 4: Three common soft tissue sarcomas compared: alveolar rhabdomyosarcoma (ARMS), embryonal rhabdomyosarcoma (ERMS), and non-myogenic soft tissue sarcoma (NRSTS). Note that chromosome 10 and 11 abnormalities are very common in ERMS but uncommon in ARMS, and virtually absent in NRSTS. 11p anomalies in ERMS are well known; the chromosome 10 segmental loss is not.

Functional Consequences of Structural DNA Alterations

Although a direct correspondence between structural alterations like copy number changes, sequence alterations, and translocations in the sarcoma genome and functional consequences is not evident at this point, a number of functional alterations are readily apparent. Two obvious examples are tumor-specific gene expression profiles and tumor-specific gene transcripts.

Gene expression profiling for tumor diagnosis and characterization has been in use for the past decade. Many hundreds of publications attest to the diagnostic utility of the technique, including sarcoma diagnosis and sub-type characterization (including definition of molecularly homogeneous subtypes of ARMS that differ from histopathologically defined ARMS. Instead, they correspond precisely with the presence of a functional chimeric gene, indicating that these chimeric genes impose a characteristic and reproducible pattern of gene expression that can be linked to specific sarcoma types. Using this information, it is straightforward to diagnostically classify such tumors, as shown in Figure 5.
However, the more important observation is that these genes are almost certainly responsible for some aspects of the tumor-type specific dysfunction, such as growth rate, treatment resistance, metastatic potential, and susceptibility to specific therapeutic agents. A major focus of current investigations is to deconvolute these gene expression patterns into biologic pathways, where tumor-specific therapeutic interdiction of the dysfunctional pathway might be possible. One specific example of this is IGF1R expression in Ewing sarcoma (and several other sarcomas); several IGF1R specific inhibitors are now available from pharmaceutical companies, and are being used in phase I and II clinical trials. However, most of these trials are not profiling patients prior to therapy in order to correlate response with IGF1 or IGF1R expression levels, nor are they attempting to integrate downstream pathway target analysis with clinical response. Given the highly variable level of expression of IGF1 ligand and receptor within a given tumor group (Figure 6), this would appear to be a lost opportunity to relate response to IGF1R inhibitors with IGF1 mediated signaling.

Figure 5: Left panel ranks overall similarity (red) and dissimilarity (black) of gene expression profiles for 3 types of soft tissue sarcoma: alveolar RMS, embryonal RMS, and non-myogenic soft tissue sarcomas.

Figure 6: Comparative expression levels of the IGF1 ligand (left panel) and IGF1R receptor (right panel) (same log scale on y axis for both) for 8 sarcomas classes: 1) PAX3 ARMS, 2) PAX7 ARMS, 3) PAX neg ARMS, 4) ERMS, 5) EFT, 6) OS, 7) Wilms, 8) NB. Note that expression levels vary by tumor type, and show significant heterogeneity within a tumor type. This is particularly marked for OS, where receptor levels are high (and variable), and ligand expression level is low and less variable. In contrast, EFT ligand and receptor levels are overall lower but more consistent with one another.
While multi-gene profiles have proved useful for tumor diagnosis and prognostic classification, a more detailed analysis of gene expression by specific tumor type has not yet gained attention. Here we refer to alternate splicing of genes. It is now well documented that the 20,000 or so annotated protein encoding genes are generally expressed in multiple splice variants. Some are tissue specific. It is therefore not surprising to expect some may also be tumor specific. We have explored this possibility, and find that there are many examples of tumor type specific isoforms of given gene. One particularly striking example is Drebrin, a developmentally regulated actin binding gene involved in neuronal growth in the brain that is known to possess at least two splice variants. In reality, comparison of multiple databases like RefSeq and AceView would suggest there are at least five documented splice variants (figure 7). Interestingly, while Ewing sarcoma is the only common childhood sarcoma to express Drebrin, the splice variant expressed by Ewing sarcoma does not match any known splice variant of this gene (Figure 7). We thus conclude that this is a unique splice variant found only in Ewing sarcoma to date (43 of 43 cases), and thus may represent an important diagnostic tool for the specific diagnosis of this tumor. It is also intriguing to speculate what biologic function, if any, this tumor-specific isoform may possess, as it is clearly a truncated transcript compared to any known splice variant, lacking the first and last three exons.

Figure 7: Drebrin expression in Ewing sarcoma and 11 normal tissues, using Human Exon arrays that probe all known exons of more than 20,000 annotated genes. Drebrin, normally expressed in developing brain (but not mature brain) is composed of 14 expressed exons expressed from the negative strand (right to left in this diagram). This is schematically portrayed in blue (RefSeq) at the top of the figure. Multiple shorter transcripts are also predicted in the AceView database (gray lines). The Ewing sarcoma transcript, found in all Ewing sarcomas studied to date but no normal tissue, appears to be yet another splice variant lacking the first and last three exons that is not previously reported.
High Throughput ‘Next Generation’ Genomic Profiling of Sarcomas

While targeted genomic profiling as shown in figures 4-7 have proven invaluable in identifying important tumor specific genomic alterations, it is now widely recognized that these methods can by definition only detect pre-determined genomic targets. In contrast, agnostic methods that interrogate every nucleotide, either for sequence determination, copy number, methylation state, or transcriptional activity offer the prospect of identifying completely novel genomic information. This is currently the focus of many cancer genome projects, largely focused on the common adult cancers, using high throughput ‘next gen’ DNA sequencing methods. Little work on childhood cancer has been announced, and none on childhood sarcomas. We have, through the auspices of Children’s Oncology Group and supported by a grant from the Department of Defense, undertaken a pilot project on Ewing sarcoma, in collaboration with Helicos, Inc., a biotech company in Boston, MA that has developed a novel ‘sequence by synthesis’ high throughput technology applicable to both DNA sequencing and ‘digital gene expression’. Figure 8 illustrates a novel approach to total genomic profiling, in this case illustrating the integrated analysis of RNA transcripts, methylation status, and DNA CNV and LOH using a combination of ChIP on Chip methylation analysis, direct methylation sequencing, Human Exon Array expression data, Digital Gene Expression data, and DNA copy number and allelic loss/gain data derived from SNP 6.0 arrays, all on a pair of cell lines from a single Ewing sarcoma patient derived from the primary, pre-treatment tumor and a subsequent chemo-resistant metastasis, as well as normal genomic DNA derived from bone marrow. As Figure 8 illustrates, a number of novel observations are evident from even a superficial inspection of the data: alternate splice transcripts, unexpected CpG islands not associated with 5’ promoter regions, non-coding RNA transcription in ‘gene desert’ regions of the genome, and a multitude of DNA copy number alterations. Clearly, a detailed analysis of integrated data sets like these will enable a far superior analysis of genomic alterations that in aggregate contribute to the malignant phenotype. These analyses have only begun and it is still too early to predict in detail, but as a general observation, the number and character of genomic alterations, both structural and functional, will likely exceed any described to date. Hopefully, instead of ‘data paralysis’, increasing sophistication and detailed analysis of these data sets will reveal true ‘driver’ defects that, when considered as a whole, define the character and clinical behavior of a given sarcoma type, and even the individual sarcoma patient.

Figure 8: Missing
Figure 9: Composite genomic data from a ~4,000 base region of chromosome 7 in Ewing sarcoma. Here, RefSeq and AceView genes are depicted at the top, and below them, in order, are Conserved sequences, Digital Gene Expression (Helicos), RNA sequence data (Helicos), HuEX total expression data, HuEx-positive strand, HuEx-negative strand, CpG island (database), 5Me Chip data, and MethSeq data (Helicos). Note that transcriptional activity in conserved regions is abundant, as expected (DGE), but abundant additional transcriptional activity is seen in non-conserved regions as well (RNASeq). Further, transcription proceeds from both strands (HuEx pos vs. neg), and while much of this correlates with CpG islands, although MethChip and MethSeq data clearly document additional methylation sites beyond these known CpG islands. In general, the picture is far more complex than traditional genomic profiling visualizes. Figure derived from analysis performed by Dr. Jonathan Buckley, based on composite data as noted in text.

Final Note

While the preceding text may raise more questions than it answers with regard to the molecular pathogenesis of sarcomas, it hopefully also provides some insight into the most promising path forward, built on multiple historical observations, now greatly enhanced with newly available ‘next gen’ DNA, RNA, and 5Me DNA sequencing and digital quantification methods that, when considered as a whole, provide an unprecedented view of the sarcoma genome. A true understanding to the pathogenesis of sarcomas will hopefully emerge as a result.

References
Gene expression profiling of soft tissue sarcomas

Isabela Werneck da Cunha

Abstract
Soft tissue mesenchymal tumors represent a group of neoplasias with different histological and biological presentations varying from benign, locally confined to very aggressive and metastatic tumors. The molecular mechanisms responsible for those differences are still unknown. Several studies have been reported trying to identify genes that can discriminate histological subtypes of sarcomas, as well as genes responsible for different biological behavior between them. Using 102 tumor samples representing a large spectrum of these tumors, our group performed expression profiling and defined differentially expression genes that are likely to be involved in tumors that are locally aggressive and in tumors with metastatic potential. Searching for molecular signature in mesenchymal tumors, we identified trios and pairs of genes capable to discriminate histological groups between all cases analyzed.

Introduction
Soft tissue tumors are a heterogeneous group of mesenchymal tumors (MT) with diverse histological presentation and clinical behavior. Based on the last WHO histological classification, there are more than 50 subtypes of MT, based upon their cellular differentiation and morphological findings. According to their biological behavior, they can be grouped into three major categories, benign mesenchymal tumors (BMT), tumors with local aggressiveness but with no metastatic potential and, sarcomas (malignant mesenchymal tumors - MMT) that have both local aggressiveness and metastatic potential. The latter group can be further subdivided as low, intermediate or high grade tumors according to NCI and FNCLCC classifications. The NCI system uses a combination of histological type, cellularity, pleomorphism and mitotic rate. The FNCLCC system is based on a score by evaluating three parameters; tumor differentiation, mitotic rate and amount of necrosis. The score is attributed independently to each parameter and the grade is a result of its adding.

At molecular level, sarcomas can be characterized by the presence or absence of tumor-specific mutations. For instance alveolar rhabdomyosarcomas are characterized by t(1;13) (PAX7;FKHR) or t(2;13) (PAX3;FKHR) translocations whereas synovial sarcomas have specific t(X;18) (SSX;SYT) translocation. In contrast, leiomyosarcomas and pleomorphic sarcomas lack specific chromosome alterations.

Sarcomas represent approximately 1% of adult malignancies but, despite this low incidence, are often of poor prognosis, at a discrepancy with their benign counterpart such as lipomas and leiomyomas that are usually well circumscribed tumors, with no local aggressiveness and without metastatic potential. In between these two extremes, there are some subtypes of mesenchymal tumors that have characteristics of both groups. They are locally aggressive but lack metastatic potential. One classical example is desmoid tumors, also known as desmoid-type fibromatosis (DTF). Whereas tumor size and histological features are the best prognostic factors available for mesenchymal tumors, little is known about molecular alterations that could contribute to the understanding of cell origin, malignant transformation, and tumor biology. Also, few molecular markers were identified as having diagnostic and prognostic values.

Gastrointestinal stromal tumors (GISTs) are one of the few successful examples of mesenchymal tumors in which the molecular events related to malignant transformation are well established. These tumors usually have an activating mutation of C-Kit gene and can be treated with imatinibe mesylate, a tyrosine kinase inhibitor. TLE1 is...
Another example of a gene that was recently described as a sensitive and specific immunohistochemical marker for synovial sarcoma. TLE proteins are transcriptional corepressors that inhibit Wnt signaling, and play a role in repressing differentiation. Measurement of TLE1 expression might have applications for diagnosis and, eventually, for the understanding of tumor biology.

Several studies using microarray technology had been reported mainly to describe gene expression signature associated to histological differentiation or outcome in specific histological subtypes.

Allander et al. described a set of genes capable of discriminating synovial sarcoma from pleomorphic sarcoma and fibrosarcomas, and also genes that differentiated monophasic synovial sarcoma from the biphasic ones. Nielsen et al. and Segal et al. also showed that synovial sarcomas, GISTs, clear cell sarcomas, mixoid liposarcoma and MPNST have a particular gene expression signature in contrast with pleomorphic sarcomas, leiomyosarcomas, fibrosarcomas and non-myoid liposarcomas. Baird et al. reported the biggest series about gene expression in sarcomas, studying 185 cases. Their results were in agreement with the former ones, showing that there are some types of sarcomas with a distinct molecular profile, and other not.

Among STT, synovial sarcoma represents a diagnosis challenging, since it shows histological features similar to others spindle cell tumors such as MPNST, fibrosarcoma, PNETs and solitary fibrous tumor.

Using a cDNA platform representing 4608 genes, our group searched for genes signatures that discriminate synovial sarcomas (14 samples) from other histological subtypes of STT (51 samples including: fibromatosis, fibrosarcoma, leiomyosarcoma, liposarcoma, MPNST, GIST, and others). cDNA microarray results pointed a set of genes that discriminate synovial sarcomas from the other histological types of STT analyzed. In our analysis, among differential expressed genes identified, the gene pairs FZD1/PLOD2, TLE-1/PLOD2 and Enthrin B3/PLOD2 precisely discriminates synovial sarcomas from the remaining samples. Validation of differential expression was done by immunohistochemistry and quantitative RT-PCR. Our results indicated that FZD1/PLOD2, TLE-1/PLOD2 and Enthrin B3/PLOD2 gene pairs represent potential molecular markers for the diagnostic of synovial sarcoma. Together or individually, all these genes could precisely discriminate synovial sarcoma from others mesenchymal tumors.

Fibroblastic tumors can range from benign tumors up to high grade sarcomas. Desmoid-type fibromatoses (DTF) is allocated between these two extremes and can be defined as clonal tumors with fibroblastic proliferation and local aggressiveness but no metastatic potential. Though, using a cDNA platform representing 4608 genes, we sought for genes that could discriminate desmoid-type fibromatosis from fibrosarcomas. Among differential expressed genes selected for validation, HEY2 (hairy/ enhancer of split related - YRPW - motif 2) was able to separate precisely DTF from fibrosarcomas. QRT-PCR and Immunohistochemical results corroborate with cDNA data with DTF showing higher expression levels of Hey2 than fibrosarcomas.

Little is known about gene expression and biological behavior in sarcomas. Ren et al. found a group of genes that can discriminate leiomyosarcomas from other sarcoma subtypes. These authors also found genes that allow the separation with different histological grades and genes related to metastatical potential between these tumors.

In an effort to identify genes that could be implicated in aggressiveness and/or metastatic behavior of sarcomas, our group compared the expression profile of set of 102 samples representing benign mesenchymal tumors, desmoid-type fibromatosis, and sarcomas. We described a set of 12 genes showing opposite expression when these two conditions were compared. SNRPD3, MEGF9, SPTAN-1, AFAP1L2, ENDOD1 and SERPIN5 were related to local aggressiveness while ZWINTAS, TOP2A, UBE2C, ABCF1, MCM2 and ARL6IP5 were related to metastasis. These genes are mainly related to cell-cell and cell-ECM interactions and cell proliferation and might represent helpful tools for a more precise classification and diagnosis as well as potential drug targets.
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Soft tissue sarcoma of the extremities

J.C. Barbe Goncalves

Abstract
The control of soft-tissue sarcomas is still evokes study and discussion. The clinical and hystopathological diagnosis is a necessary base to planning surgical treatment (limb sparing), radiotherapy and chemotherapy. This paper aims to evaluate the diagnosis, staging and treatment and correlated with outcome.

Introduction
Soft tissue sarcoma is a heterogeneous group of malignant tumors. There are embryologically derived from mesoderm. Tumors of high and low grade, with potential for local recurrence and distant metastasis, can be found in the subcutaneous and deep regions in the limb, and often located in a single compartment.

Incidence
In 2005 registered about 3.2 cases per 100,000 soft-tissue sarcoma, representing 1% of malignant tumors diagnosed and more frequent in men 1.42:1. It may occur at any age and is more common in adults. 15% occurs in patients with less than fifteen years and 40% in patients over 55 years. They account for 0.61% of cancer deaths in 2005 in USA (1). The soft-tissue sarcomas are a heterogeneous group with more than 50 subtypes.

The incidence of subtypes is age dependent. Rhabdomyosarcoma occurs only in patients of lower age, predominantly undifferentiated and pleomorphic sarcoma occurs in patients of old age, while sinoviossarcoma are more frequent in younger patients. They can be found anywhere in the body, 60% in extremities, 20% in trunk, 15% retroperitoneum and 5% head and neck. 1/3 of the soft tissue sarcomas occurs in subcutaneous (2).

Epidemiology
More than 50 distinct subtypes of soft tissue sarcoma have been reported, they behavior have tendency to be similar. Prognosis is dependent on the size, histological grade, and depth in soft-tissue (3). The most common sarcoma of soft tissues is the high-grade pleomorphic undifferentiated. The spread of metastases occurs preferentially by hematological way. Probable ionizing radiation, chemical and physical agents and immunologic defects of P53 may be what causes in developments a soft-tissue sarcoma.

The most common tumor after irradiation is the pleomorphic undifferentiated in 70% of cases, followed by osteosarcoma, and fibrosarcoma. Usually the long-term survival of sarcoma after irradiation is low with reserved prognosis, and the survival at 5 years are 10% to 30% (4).

Other rare situation that causes soft tissue sarcoma are, in sites of metal implants and the use of herbicides. The percentage of P53 mutation is observed in approximately 30 -60% of soft-tissue sarcomas. The deletion of the P53 is found in Li-Fraumeni syndrome, resulting in a familial rhabdomyosarcoma, breast cancer and others. Chromosomal alterations inherited or acquired, has been described as causes of soft-tissue sarcoma (3).

The soft-tissue sarcomas are a group of tumors arising from connective tissues, with the capacity to invade adjacent tissues, with a tendency to recurrence and metastasis. However in some cases we see the exception, like of dermatosarcoma protuberans with low potential for metastasis. However, high-grade pleomorphic undifferentiated STS often metastasizes to the lung by hematogênicroute and angiosarcoma, epithelioid sarcoma, synovial sarcoma, and rhabdomyosarcoma, have
preferred spread bylymphatics (3).

Classification

On the staging of any tumor, it is necessary to include it in treatment protocols. It is a process that using imaging methods in combination with histopathology. Depending on the histological grade, location of tumor in imaging exams, it could be included in a grade of staging system and it could be included in a protocol of treatment, generating a prognostic factor.

The advancement of imaging examinations and identification of pathology, contributed to the evolution of the staging, biopsy, surgical planning, radio and chemotherapy. The staging systems are the most widely used classification of Enneking and American Joint Committee on Cancer (AJCC) (Table 1).

Table 1: Stage Grouping

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
<th>G</th>
<th>Grade</th>
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<tr>
<td>I A</td>
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<td>NX</td>
<td>M0</td>
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<tr>
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<td>T2b</td>
<td>N0</td>
<td>NX</td>
<td>M0</td>
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<tr>
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<td>N0</td>
<td>NX</td>
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</tr>
<tr>
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<td>Any T</td>
<td>N1</td>
<td>NX</td>
<td>M0</td>
<td>Any G</td>
</tr>
<tr>
<td></td>
<td>Any T</td>
<td>Any N</td>
<td>M1</td>
<td>Any G</td>
<td>Any G</td>
</tr>
</tbody>
</table>

The study of the imaging examinations begins with plain radiography, which can identify the significant mass volume, calcifications (synoviosarcoma), bone invasion, cortical irregularities. (Fig 1).

The ultrasound in soft-tissue sarcoma, is used to show the depth of the lesion, consistency, content, and can be used to perform the biopsy. CT scan is an examination of choice for assessment of bone structure, matrix remodeling and tumor (Fig. 2). The CT scan was used as the primary method of staging, and it can show the pulmonary metastases.

Figure 1: Plain radiograph shows a congenital fibrosarcoma, shows growing soft-tissue mass in the posterior region of the thigh.

Figure 2: CT of the thigh shows heterogeneous expansive lesion near the bone.
The bone scintigraphy using tc99m, has limited use for assessment of soft tissue sarcomas. It is rarely used for staging, but it is good to assess skeletal involvement by the tumor.

MRI is a particular method of choice for staging of soft-tissue sarcomas, with multiplanar acquisition system, it better defines the lesion and its adjacent tissue involvement with making the pre-operative planning, defining the surgical limits, safely with reduced morbidity. (Fig 3)

Figure 3: shows the MRI with tumor extension on the thigh.

The Pet-CT access the metabolic activity of the tissue with the potential to differentiate a benign lesion from other malignant, also being used to evaluate residual disease. It is a modality of choice for the diagnosis in pulmonary metastases with less than 0.5 cm and efficient to detect metastasis in lymph nodes smaller than 1.0 cm (5).

Soft-tissue mass, often painless, fast-growing, in few months and other times grow slow for several years; this behavior apparently depends on the histological degree of the tumor. (Fig. 4).

Figure 4: shows the patient 6 months of age with congenital fibrosarcoma of thigh with rapid growth (3 months), apparently painless.

The tumor should be submitted on physical examination by determining if it is superficial or deep, if it is fixed or mobile in the deep planes, as well as consistency, temperature and local lobules. The regional lymphatic nodes should also be investigated.

The limb salvage surgery is the first choice for treatment of the extremity soft tissue sarcoma. Amputation surgery is indicated, for recurrent tumors, or inadequate resection with positive margins. The goal of surgical treatment is a low rate of recurrence in limb salvage and in amputation.

The tumor should be resected with a wrapping of the healthy tissues. If adequate margins cannot be achieved, then the amputation should be considered. Usually the tumors of soft tissue, are attached to bone, if it is not invading the cortical, a subperiosteal resection give adequate oncologic margin. (Fig. 5 a and b).

Figure 5a: shows the tumor surrounded by healthy tissue.

Figure 5b: shows the periosteum with the resected specimen.
The Istituto Nazionale per lo Studio e la Curazione dei Tumori, Milano, Italy, evaluated for 20 years, 911 patients for surgical margins and prognosis. Defined as positive margin, those with less than 1mm and negative with more than 1mm. 642 patients had primary tumor, and 269 were postoperative recurrence. 711 specimens were considered as positive and 163 negative. The following average was 107 months. Despite the prognostic value adding tumor size, depth and histological grade, the margin was not statistically significant for prognosis; with clear trend to a better prognosis tumors with negative margins (6).

The superficial tumors smaller than 5 cm high or low grade must be removed, the largest 5 cm deep and should be resected, with previous biopsy and followed by radiotherapy. The chemotherapy is still discussed and it is used for large high-grade tumors.

The biopsy should always be done on the surgical incision and executed by experienced surgeon, and if possible to freeze the biopsy specimen to assess the quality of tissue obtained. It can be done by needle, and incisional or excisional (Fig. 6a and 6b), the hemostasis should be rigorous to prevent hematomas and extracompartimental involvement, avoiding the resection with limb salvage procedure.

Figure 6a, shows with a needle biopsy, and Figure 6b, excisional biopsy.

A needle biopsy can be done with the aid of CT and ultrasound, with the possibility of removing the specimen for a more heterogeneous area and assist the pathologist.

Avoid to make a transverse incisions on the limb or the site of biopsy adjacent to the vessels and nerves (Fig. 7).

Figure 7: shows transverse scar incision upper limb of a child at the time of reresection of a Ewing sarcoma with positive margins.

The Skeletal Muscle Tumor Society (MSTS), provides four types of resection, such as intalesional (intra tumoral), marginal, (removes the tumor in pseudo capsule), Wide (remove a cuff of healthy soft tissue around the tumor), ideal for local control of soft-tissue tumors, and radical (the resection of the entire compartment).

The tumor size and location are two important factors influencing the resection. Bump at the surface should be resected if symptomatic. With large lesions, biopsy should be before the resection.

Radiotherapy

Radiotherapy is often used for the treatment of soft tissue sarcomas. When used after the surgical negative margin resection, local control over the long term results is around 90%.

The aim of radiotherapy is to associate surgery to promote a tumor local control. The late effects of radiation are dependent to the dose and the volume of irradiated site.
Methods of radiotherapy:
1. Brachytherapy
2. IMRT Intensity Modulated Radiation Therapy
3. Proton-Beam Radiation Therapy
4. Fast neutron and Carbon Ion Radiation Therapy

In Denmark a study was done for evaluation patients who underwent limb salvage surgery, and adding to the site abrachytherapy combined with external radiotherapy, for evaluating the local control and prognosis. They evaluated 39 patients who underwent tumor resection with limb sparing, associatebrachytherapy (20Gy) and external beam radiotherapy (50Gy). The patients were followed for a 9 years period. After this, study concluded that patients who undergo the brachytherapy and external radiation had better local control of the lesion, with less toxicity and lower local side effects of radiation.

Figure 8: The placement of catheters for brachytherapy after resection of fibrosarcoma.

The final dose of radiation can be used for the treatment of soft tissue sarcomas. However a study published in 2005, evaluates the results of high doses of radiotherapy in patients who were not treated with surgical resection. The study was conducted Massachusetts General Hospital, were 112, patients (43% of extremities), with soft tissue sarcomas large volume and high grade. The doses above 68 Gy, were considered therapeutic and better local control of tumor and disease-free survival, although this dose begin to increase the complications consequent to radiotherapy. It is likely that with the use of IMRT and Proton Beam radiation therapy may reduce these complications in the future.

Patients where conservative surgery resulted a dysfunctional limb, radiotherapy should be considered. In tumors with 10cm or above, the use of radiotherapy at doses over 63 Gy, give local control in a long term less than 25%. Other study compared radiotherapy before and after limb sparing surgery, and concluded that after the 3 years that followed was no difference between the two groups, but patients who received pre operative radiation had more complications in the surgical wound. Otherwise patients who received postoperative radiation had more late complications such as fibrosis, edema, and fracture. They recommend that a gold standard, for treatment of soft tissue sarcoma, should be done with surgery followed by postoperative radiotherapy. The control of local recurrence is more effective.

Chemotherapy

The soft-tissue sarcoma are usually treated with surgery and pre and post operative radiotherapy. The surgery to preserve the limb and radiotherapy has a good local control at 5 years, but the patients going to death by metastatic disease. The significance of chemotherapy in the treatment takes place for the control of micrometastases. The difficulty in controlling the tumor by chemotherapy is the fact that the soft tissue tumors divided in subtypes and can not design efficient protocol compared to the appropriate treatment protocols for bone sarcomas. The cytoreduction is particularly important to reduce masses and provide limb sparing and controlling distant micrometastases although there is no general consensus for the use of chemotherapy for the treatment of sarcomas of soft tissue.

The protocol is based on doxorubicin and ifosfamide, with questionable results, and it not be considered as a treatment of choice in high-grade sarcomas resected surgically. Recent studies report the beneficial effects of these drugs. The sinutinib, inhibitor of growth factor, in combination with radiotherapy, it is effectively reducing cell growth and vascular income of the tumor. Enhance radiotherapy for the control of soft tissue sarcomas in genetically produced in animals.

The local control and metastasis from soft-tissue sarcomas is not satisfactory with chemotherapy,
perhaps in the near future with development of new chemotherapy drugs, we can improve local and distant control of high-grade and metastatic soft-tissue sarcomas.

Conclusion
The local control of soft-tissue sarcoma becomes improved with association and development between surgical staging, limb salvage surgery, and radiation therapy, but some patients dead because lack control of metastasis.

The search for a systemic efficient treatment should be done, and probably the control will be at the molecular therapy, with the advent of new agents that have specific targets.

References
3. Gilbert, NF; Cannon CP; Lin PP, Lewis VO; Soft-tissue Sarcoma: Journal of the American Academy of Orthopaedic Surgeons; Volume 17, Number 1, January 2009 40-47.
Abstract

Rhabdomyosarcoma (RMS) represents the most frequent type of soft-tissue sarcoma in childhood and adolescence. Radiotherapy as part of a multimodal therapy approach plays an important role in the management of pediatric RMS. Indications for and doses of radiotherapy depend on risk factors like postsurgical stage, age, histology, tumor size, and tumor site. New technical radiotherapy approaches like intensity-modulated radiotherapy or proton therapy have been introduced to decrease the potential late sequelae of radiotherapy. However, little is known regarding the clinical use of these techniques. Due to the young age of most patients with risks of severe late sequelae, radiotherapy in pediatric RMS patients remains a sophisticated therapy approach.

Introduction

Rhabdomyosarcoma (RMS) represents the most frequent type of soft-tissue sarcoma in childhood and adolescence. RMS may occur in any location within the body, and may metastasize to lungs, bones, bone marrow, lymph nodes, and other sites. Several histologic subtypes like embryonal RMS, botryoid and spindle cell RMS, and alveolar RMS are described (13). A common classification of the Intergroup Rhabdomyosarcoma (IRS) group distinguishes four different patient subgroups according to postsurgical stage: Group I: patients with a complete excision of localized disease; Group II: patients with localized disease (with or without regional lymph node tumor) that was grossly removed with microscopic residual disease; Group III: patients with localized gross residual disease; Group IV: patients with distant metastases at diagnosis. The IRS-I, II, and III reports (2,9,10) showed that patient outcome was associated with group classification. In an analysis of patients with localized RMS treated within the Cooperative Weichteilsarkom Studiengruppe (CWS)-81, -86, -91 and -96 studies, age, histology, tumor size, tumor site, postsurgical stage, and omission of radiotherapy were identified as factors associated with an increased relapse risk (3). Radiotherapy indications and doses are therefore adapted to IRS groups and other risk factors.

Indications for radiotherapy

The value of radiotherapy in patients with IRS group I tumors has been characterized in the IRS trials I, II and III (17). Radiotherapy was randomized in the IRS-I trial, no radiotherapy was given in IRS-II trial and radiotherapy was applied in patients with alveolar histology only in the IRS-III trial. Patients suffering from alveolar RMS showed a significantly improved failure free as well as overall survival (82% vs. 52% after 5 years) if they had been treated with radiotherapy. For patients with embryonal RMS, a (non-significant) trend towards increased failure free survival without any difference in overall survival (about 95% after 10 years) was observed (18). In conclusion, patients with alveolar RMS IRS group I benefit from radiotherapy, but patients with favorable histology do not.

Schuck et al. (15) analyzed patients with IRS group II RMS and RMS-like tumors treated in the CWS trials -81, -86, -91 and -96. Favorable subgroups of patients had not been treated with radiotherapy. However, the indications for radiotherapy differed within these trials. Despite a potential selection bias, patients treated with radiotherapy showed a statistically significant improvement in local control (83% vs 65%, p<0.004) and event free survival (76% vs 58%, p<0.005) 5 years after treatment in comparison to those treated without radiotherapy. There was a trend for improved survival in the radiation group (84% vs. 77%, n.s.). Improvement in local control and event free survival was independent of established risk factors (histology, size, site,
Therefore radiotherapy is widely applied in patients of RMS IRS group II. Due to the absence of a significant impact on the survival, radiotherapy can be omitted in patients with favorable risk factors if radiotherapy is considered as too toxic, e.g. in very young children.

In IRS group III patients radiotherapy is the only available local therapy if there is no chance for a secondary complete resection. Therefore radiotherapy is indicated in almost all IRS group III RMS patients. If a delayed secondary resection is possible and complete resection (R0) is achieved, patients still benefit from additional radiotherapy. In IRS group III patients there are only few situations that lead to an acceptable outcome after an omission of radiotherapy, e.g. in young children with small tumors and favorable histology that show a complete remission to initial chemotherapy or underwent a secondary complete resection. An example for the omission of radiotherapy could be (usually very young) patients with vaginal RMS and complete remission after chemotherapy (1).

A special challenge is the treatment of very young patients. The general radiotherapy indications as stated above have to be adapted due to the very high risk of severe late complications. Therefore an individual decision for or against radiotherapy must be made depending on tumor histology, tumor site and size, response to chemotherapy, extent of previous resections and options for secondary resection (12).

Target volume definition

The target volume typically includes the initial tumor volume with an additional security margin of 1.5 cm for the clinical target volume (CTV) as well as an additional margin of 0.5 cm for the planning target volume (PTV). The target volume definition refers to the pre-therapeutic T1 magnetic resonance image with contrast in most cases. In extremity tumors, larger security margins may be used in cranio-caudal extension. Surgical scars from biopsies/resections as well as drain sites have to be included into the target volume. Smaller safety margins have to be used if otherwise non-invaded critical structures beyond well-defined anatomical borders (e.g., eye, optic chiasm) would be irradiated. Pelvic or chest wall tumors with non-infiltrating extension into pre-formed cavities often show an enormous shrinking under chemotherapy. Irradiating the pre-treatment volume would mean that large volumes of healthy tissue (bowel or bladder in the pelvis, lung in case of chest wall) were included in the radiation field. In these cases, the target volume in the areas of non-infiltrating tumor may encompass only the residual mass at the beginning of radiotherapy and a 2 cm safety margin. For all other parts of the tumor (infiltrated muscle or bone) the earlier mentioned more extended safety margins are to be applied. There are some further special sites (e.g. orbit, genito-urinary tract) that require an adjustment of the target volume with individual risk-benefit considerations.

Radiation doses

In general, a normofractionated radiation dose of about 50 Gy is supposed to be sufficient for alveolar RMS independent of remission status as well as for embryonal RMS with residual disease following induction chemotherapy without an option for secondary resection. However, a further boost using a shrinking field technique may be used in selected patients who are supposed to be at high risk. In the IRS-IV trial, radiotherapy doses of 50.4 Gy (28 1.8-Gy daily fractions) were randomized against 59.4 Gy (54 1.1-Gy twice daily fractions) in patients with group III tumors (5). No differences in failure-free or overall survival were found. In IRS group III patients with favorable histology and a clinical complete remission without the option of a complete secondary resection, radiation doses of 32 Gy (20 1.6-Gy twice daily fractions) have resulted in satisfactory local control in the CWS trials (7). Conventional fractionated doses of 40 Gy or more have been reported to be sufficient to obtain local control in these special cases (13,15). This dose can also be assumed to be sufficient in IRS group I and II patients.

Radiation technique

Generally megavoltage equipment (4-20 MV linear accelerator) is recommended with computed tomography based 3-dimensional treatment planning. Special sites may require new technical radiotherapy approaches to reduce the radiation doses to the surrounding healthy tissue. Several reports describe the use of brachytherapy (e.g. in orbital or genito-urinary tumors) (8), intensity-modulated radiotherapy (IMRT)
(e.g. parameningeal RMS) (18), or proton therapy (16). However, very little data is available regarding the clinical use of any of these techniques. IMRT and the passive scattering technique of proton therapy may be associated with an increased risk of secondary malignancy induction (6). Therefore, the use of these techniques should be restricted to situations in which any other technique cannot result in a satisfying treatment plan. Proton therapy with an active scanning technique seems to be promising in the future (16). However, there are still many problems of availability in many countries.

Stage IV patients
Metastases seem to have a similar response to radiotherapy as the primary tumor. The decision for radiotherapy of metastases has to be taken according to an interdisciplinary concept for treatment and should follow the rules for irradiation of the primary tumor. Radiation doses should be adapted to the tumor histology and the individual situation of the patient. In palliative treatment situations, radiotherapy should be restricted to sites that cause (or may cause in future) symptoms like pain, bone instability, organ obstruction etc.

Outcome
The outcome of RMS patients has been significantly improved during the last decades. In an analysis of 1164 patients with non-metastatic RMS who achieved complete remission at the end of multimodal therapy in the CWS-81, -86, -91, and -96 trials between 1980 and 2002, 337 patients developed either locoregional, metastatic, or combined relapses (median follow-up, 5 years). Five-year overall and event-free survival after diagnosis for all patients was 77% and 67%, respectively. For relapsed patients, 5-year postrelapse survival was 24% (3). In children with IRS-group III RMS treated within the IRS-4 trial, Donaldson et al. (5) reported a 5-year failure-free survival rate of 70% and an overall survival (OS) of 75%.

Side effects
Due to low cure rates in former times therapy optimization approaches have mainly been focused on survival improvement for long times. Nowadays, with the increases in cure rates also a higher frequency of therapy-associated late effects among the rising amount of survivors occurs. As radiotherapy may be necessary in any part of the body, radiotherapy-associated late sequelae may also arise in almost every organ. A review about frequent side effects was published by Dieckmann et al (4). For RMS patients, special concerns arise in the treatment of parameningeal tumors. In a series of 90 patients who had been treated with radiotherapy in this location, Meazza et al. (11) found high rates of facial growth retardation (72%), dental abnormalities (72%), trismus (41%), xerostomy (38%), growth hormone deficiency (44%) and other problems during late follow-up. New treatment approaches have been introduced to try to reduce these risks (18). However, long-term results have not been reported.

Summary
Radiotherapy as part of a multimodal therapy approach plays an important role in the management of pediatric RMS patients. Radiotherapy is used in all alveolar cases and in Group II to IV embryonal histology. Radiation doses reach from 32-50 Gy differentiated according to risk factors like postsurgical stage, histology, size and site. Using this approach high cure rates can be achieved in localized disease.

References
5. Donaldson SS, Meza J, Breneman JC, Crist WM, Laurie


Particularities on diagnosis and management of non-rhabdomyosarcoma soft tissue sarcomas

Andrea Ferrari

Abstract
The definition of non-rhabdomyosarcoma soft tissue sarcomas includes a varied group of malignant soft part tumors that can occur in childhood, but the majority of which are entities typically seen in adult age. Like their adult counterparts, they are usually considered scarcely sensitive to chemotherapy, but treatment strategies for these tumors have changed to some degree in recent years, and multiple-modality treatments that also include chemotherapy have increasingly been attempted. Subsets of patients with specific histological subtypes and prognostic variables have been thought likely to benefit of chemotherapy. The recent development of new molecular treatment approaches to specific tumor targets may enable the current limits of systemic therapies to be overcome in the near future, possibly identifying specific agents tailored to each histotype. While awaiting these developments, however, a better use of standard chemotherapy may prove important in improving the cure rate for these patients.

Introduction
Soft tissue sarcomas (STS) are a heterogeneous group of mesenchymal extraskeletal malignant tumors, classified on the basis of their differentiation according to the adult tissue they resemble. They are rare tumors (annual incidence of around 2-3/100,000, less than 1% of all malignancies), but in pediatric age they account for 8% of all neoplasms.

While rhabdomyosarcoma (RMS) represents about half the cases of STS in childhood and adolescence, the remainder 50% of pediatric STS are the so-called “non-rhabdomyosarcoma” STS (NRSTS): this definition covers various entities with a different biology and clinical behavior, the majority of which are tumors typically found in adults, featuring a local aggressiveness and a propensity to give rise to distant metastases that usually correlates with their grade of malignancy.

Whilst in the past children with NRSTS have often been treated according to the principles adopted for RMS (which is a clearly distinct entity, characterized for example by a high grade of malignancy but also by a strong response to chemotherapy as a general rule), it has been currently recognized that they should be treated within specifically tailored clinical trials, which must draw insight also from the numerous experiences coming from adult STS.

Cooperation between pediatric oncologists and adult medical oncologists dealing with STS has been improving, even if differences in treatment strategies and in outcome still exists. It remains not completely clear, however, whether a certain histotype has the same clinical behaviour - and the same biology - when arising in adults or in children. The situation is even more complicated by the fact that STS are a variety of very different tumors, with different biology, natural history and response to treatment: the heterogeneity may be related to the histotype (the clinical course can widely vary along the different histotypes), but also to the different grade of malignancy within the same subtype (same histotypes may behave completely different according to their grade, while different histotypes with the same grade of malignancy may display the same clinical history).

Controversies on treatment strategy
Like their adult counterparts, pediatric NRSTS tend to be seen as being relatively insensitive to chemotherapy; this makes surgery the unquestionable cornerstone of their treatment, while radiotherapy may have an important part to play in local control, though the patient’s age is always an important factor to consider when considering irradiation, to keep its late sequelae to a minimum.
Treatment strategies have changed a little in recent years, however, and multiple-modality treatments including systemic chemotherapy have increasingly been attempted. There are subsets of patients (i.e. those with high-grade, large, invasive tumors), whose outcome is in fact unsatisfactory if the treatment is limited to local therapies alone, because of a marked tendency to spread. Controversies still remains, however, because contradictory findings emerged on one hand from the body of clinical trials (most of which concern adult STS) and on the other hand from the day-to-day clinical practice. For example, general conclusions of randomized trials told us that adjuvant chemotherapy is pointless or, at best, only marginally effective in adult STS, and that multiple-drug regimens (e.g. full-dose ifosfamide with doxorubicin) seem to offer no advantage over single-agent chemotherapy (doxorubicin alone) in advanced disease. But many of the negative results recorded in randomized adjuvant chemotherapy studies need to be reconsidered, because they did not use the drug combinations currently recognized as the most effective in STS nor had they selected patients most likely to respond to chemotherapy, grouping together tumors of diverse histology, grade, and size. When these criteria was strictly followed (targeting a selected group of high-risk patients and delivering a regimen of full-dose ifosfamide plus anthracyclines), the Italian Sarcoma Group (ISG) study closed its randomization in advance because of a striking improvement in the survival of patients receiving chemotherapy. As a matter of fact, however, in contrast to the results of most published studies, current clinical guidelines usually suggest adjuvant chemotherapy and polychemotherapy for most STS, also in adult setting, though in formal terms these can hardly be called “evidence-based” treatments.

One of the problem, probably, is represented by the limits of our clinical and statistical methodologies, when it comes to dealing with rare tumors (as NRSTS are), and it has been emphasized that the results of clinical trials should not become the be all and end all when decision-making with a specific patient in mind (for instance, a given patient with a large, high-grade STS may be exposed to a different risk and have a different chance of benefiting from chemotherapy from the “average patient” considered in published clinical trials suggesting little or no statistically significant efficacy of chemotherapy).

The contribution from pediatric oncologists to the debate on the role of chemotherapy in these tumors is naturally more limited. The only randomized trial performed in the pediatric age group, by the Pediatric Oncology Group (POG) between 1986 and 1992, failed to consider the benefits of adjuvant chemotherapy because the majority of patients refused randomization. But it is worth taking a look at the impressions recently emerging from pediatric studies. Firstly, they suggested that – when an adequate patient selection based on histology and on prognostic variables is done, in order to identify patients who are at high risk of metastatic failure and consequently in much need of systemic treatment, as well as those whose histological characteristics make them more likely to respond to chemotherapy - chemotherapy may have a more significant beneficial impact than is generally believed.

In the direction of target diagnostic subgroups that might be as specific and homogeneous as possible, there are: 1) the definition of “adult-type” NRSTS (pediatric NRSTS typical of adulthood, definitely malignant, and with morphological features resembling differentiated/mature tissues), excluding from the same trials and the same analyses, those biologically/clinically different entities that, studied together, give rise to misleading results – i.e. infantile fibrosarcoma, borderline tumors and small round cell tumors); 2) the idea to analyze synovial sarcoma separately (its chemosensitivity probably comes midway between that of the most typical adult sarcomas - with less than 40% of patients responding to chemotherapy - and that of pediatric small round cell tumors, such as rhabdomyosarcoma - with up to 80% of responders).

As defined in the NRSTS 2005 protocol of the European pediatric Soft Tissue Sarcoma Study Group (EpSSG), for instance, ifosfamide-doxorubicin chemotherapy is required in all but a few very-low risk (group I, tumor size < 5 cm) patients with synovial sarcoma; in “adult-type” NRSTS, neo-adjuvant chemotherapy is the first treatment to attempt for patients with advanced disease, in order to make such cases amenable to conservative complete resection, but also in promptly treating any micrometastases, while adjuvant chemotherapy is indicated in selected high-risk (high-grade, large tumors) resected patients.
Table 1: Most significant studies published on pediatric NRSTS

<table>
<thead>
<tr>
<th>Author</th>
<th>Series</th>
<th>Results</th>
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<tr>
<td>Pratt CB, 1998 POG</td>
<td>75 pts, unresected/metastatic</td>
<td>response to CT plus RT ~ 50%</td>
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<tr>
<td></td>
<td></td>
<td>4-yr EFS 18%, OS 31%</td>
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<tr>
<td>Pratt CB, 1999 POG</td>
<td>81 pts, surgically resected randomized study on adjuvant CT</td>
<td>most refused randomization - no data on the efficacy of adjuvant CT</td>
</tr>
<tr>
<td></td>
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<td>5-yr EFS 72%, OS 84%</td>
</tr>
<tr>
<td>Spunt SL, 1999 St Jude Children Research Hospital</td>
<td>121 pts, surgically resected</td>
<td>adjuvant CT given to 31%</td>
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<tr>
<td></td>
<td></td>
<td>5-yr EFS 77%, OS 89%</td>
</tr>
<tr>
<td>Spunt SL, 2002 St Jude Children Research Hospital</td>
<td>40 pts, initially unresected (group III)</td>
<td>response to CT plus RT = 58%, response to CT alone or RT alone = 20%</td>
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<tr>
<td></td>
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<td>5-yr EFS 33%, OS 56%</td>
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<tr>
<td>Ferrari A, 2005 Istituto Nazionale Tumori, Milan, Italy</td>
<td>182 pts (100 group I, 36 group II, 40 group III, 6 group IV)</td>
<td>group I-II: 51% had adjuvant CT</td>
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<tr>
<td></td>
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<td>5-yr EFS 72%, OS 86% - 5-yr MFS 35% for G3, &gt;5cm group III: 5-year EFS 45%, OS 52% response to CT = 39% for CR+PR, 58% for CR+PR+MR</td>
</tr>
<tr>
<td>Pappo AS, 2005 COG</td>
<td>39 pts, unresected/metastatic</td>
<td>response to CT (IFO-DOXO +VCR, with G-CSF) = 41%, but 30% when synovial sarcomas are excluded 3-year PFS 44%, OS 59%</td>
</tr>
<tr>
<td>Nathan PC, 2005 NCI</td>
<td>25 pts, unresected/metastatic</td>
<td>response to CT = 80% (CR+PR), but no any histotype selection 5-year EFS 34%, OS 50%</td>
</tr>
<tr>
<td>Ferrari A, 2005 ICG-CWS</td>
<td>36 pts, group I-II, high-risk (G3, &gt;5cm) synovial sarcoma excluded)</td>
<td>5-year MFS 36%, OS 37%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pts receiving CT: MFS 49%, OS 41%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pts not receiving CT: MFS 0%, OS 24%</td>
</tr>
<tr>
<td>Ferrari A, 2007 Istituto Nazionale Tumori, Milan, Italy</td>
<td>112 cases, fulfilling the criteria for the MSKCC nomogram application</td>
<td>10-yr mortality 29% comparison prognostic factors with those established in adults strong adverse effect of tumor size</td>
</tr>
</tbody>
</table>

Legend: NRSTS – non-rhabdomyosarcoma soft tissue sarcomas; POG - Pediatric Oncology Group; COG - Children Oncology Group; NCI - National Cancer Institute; ICG – Italian Cooperative Group; CWS – German Cooperative Group; MSKCC - Memorial Sloan Kettering Cancer Center; CT – chemotherapy, RT – radiotherapy; IFO – ifosfamide; DOXO – doxorubicin; VCR – vincristine; G-CSF - granulocyte colony-stimulating factor; IRS – Intergroup Rhabdomyosarcoma Study; EFS – event-free survival; PFS – progression-free survival; MFS – metastases-free survival; OS – overall survival; CR – complete response; PR – partial response; MR – minor response; pts – patients; year - yr.

Figure 1: Relative incidence of different soft tissue sarcoma histotypes in children/adolescents. Legend: RMS = rhabdomyosarcoma; pPNET = peripheral primitive neuroectodermal tumor; NRSTS = non-rhabdomyosarcoma soft tissue sarcoma; MPNST = malignant peripheral nerve sheath tumor.
### Table 2: Recent large series on pediatric synovial sarcoma

<table>
<thead>
<tr>
<th>Author</th>
<th>Series</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okcu F, 2003</td>
<td>219 pts &lt; 20 yrs</td>
<td>5-yr OS 80%, response to CT – 60% adjuvant CT did not correlate with outcome</td>
</tr>
<tr>
<td>Ferrari A, 2004</td>
<td>271 pts of all ages (46 cases = 16 yrs) comparison pediatric versus adult cases</td>
<td>5-yr EFS 37%, OS 64%, response to CT – 50% age 0-16 yrs - 78% had adjuvant CT - EFS 66% age &gt; 17 yrs - &lt;20% had adjuvant CT – EFS &lt;40% MFS correlated with the use of adjuvant CT</td>
</tr>
<tr>
<td>Brecht IB, 2005</td>
<td>Istituto Nazionale Tumori, Milan, Italy</td>
<td>5-yr EFS 77%, OS 89%, nearly all pts received chemotherapy group I, £ 5 cm (48 pts) - no metastases (identification of low-risk pts)</td>
</tr>
<tr>
<td>Ferrari A, 2008</td>
<td>150 pts &lt; 18 years with grossly-resected tumors (IRS group I-II)</td>
<td>5-yr EFS 63%, OS 77%, response to CT: 4 CR, 11 PR, ICG comparison extremity 9 MR, 7 NR main prognostic factors: IRS group, T status, size 5-yr EFS 70% for extremity, 43% for axial sites</td>
</tr>
<tr>
<td>Brecht IB, 2005</td>
<td>ICG-CWS</td>
<td>5-yr OS 89%, nearly all pts received chemotherapy group I, £ 5 cm (48 pts) - no metastases (identification of low-risk pts)</td>
</tr>
</tbody>
</table>

* Multicenter study: M.D. Anderson Cancer Center, St. Jude Children Research Hospital, Istituto Nazionale Tumori Milan, and CWS group. **Legend:** IRS – Intergroup Rhabdomyosarcoma Study (staging system); EFS – event-free survival; LDFS – local relapse-free survival; MFS – metastasis-free survival; OS – overall survival; CR – complete response; PR – partial response; MR – minor response; NR – no response; ICG – Italian Cooperative Group; CWS – German Cooperative Group; CT – chemotherapy; pts – patients; year - yr.

### Histology-driven therapy

While it was once assumed that all STS should be treated according to the same approach, efforts are now increasingly being made to explore new therapeutic options tailored to each histological subtype. Drugs other than the ifosfamide-doxorubicin combination have proved fairly active against particular histotypes, albeit in adult trials. Taxanes have been found active against angiosarcoma. Gemcitabine and, more recently, gemcitabine associated with docetaxel are effective against leiomyosarcoma. Trabectedine has recently revealed an impressive activity in myxoid/round cell liposarcoma (with a possible direct effect on the products of the histotype-specific FUS-CHOP translocation) and the potential for an effect on leiomyosarcoma and Ewing’s sarcoma too, to the point that it has been recently approved by the European Medicines Agency (EMEA) for the treatment of sarcomas.

What may be more important, however, are developments concerning new targeted therapies. Anti-tyrosine kinase imatinib mesylate has been used successfully to treat c-kit-positive gastrointestinal mesenchymal tumors (GIST), pointing to new opportunities for us to design new molecular therapies to home in on targets crucial to a given tumor’s biology. Imatinib has also been effective against dermatofibrosarcoma protubersans, presumably because it can deregulate the platelet-derived growth factor-B (PDGFB) resulting from the specific t(17,22) translocation. Preliminary data have become available on the effects of vascular endothelial growth factor (VEGF) inhibitors (on vascular sarcomas and leiomyosarcoma), and of mammalian targets of rapamycin (mTOR) inhibitors (on leiomyosarcoma).

Together with the development of target therapies, new methods for the evaluation of tumor response are emerging. Response to neo-adjuvant treatment in patients with STS has been usually evaluated according to dimensional criteria. The experience in GIST (treated with Imatinib) has showed that the changes in tumor tissue characteristics could be important indicators of therapeutic response even in the absence of tumor shrinkage, while the dimensional criterion alone can underestimate both response and progression. The "Choi criteria", proposed for GIST, considered for instance the changes in size but also in Computed Tomography (CT) density (i.e. a decrease in tumor size of more than 10% or a decrease in tumor density of more than 15% had a sensitivity of 97% in detecting patients with good response). The improvement in the imaging techniques currently permits to better detect changes in tissue characteristics (tumour density on CT scan, signal intensity on Magnetic
Resonance Imaging, contrast uptake on Positron Emission Tomography scan, functional imaging using multidetector row computed tomography, also known as perfusion CT, and dynamic contrast-enhanced MRI). Recent findings coming from adult STS would suggest that this concept may be true not only for GIST, but also for soft tissue sarcomas, and not only in case of target therapies, but also when classic cytotoxic regimens are used.

So times are changing. Nonetheless, pediatric oncologists must bear in mind at all times that the expectation that specific therapies become available soon for each NRSTS subtypes, will only come true if we continue to strive to create and consolidate a close cooperation between pediatric oncology groups around the world, and to ensure an ever better exchange of information with the adult medical oncology world. Our success in developing new weapons against NRSTS will also depend on our ability to reinforce the ties between clinicians and translational research, breaking down some of the numerous ethical, commercial and methodological barriers that often interfere with the development of new drugs specifically for pediatric cancers.

Summary

As in the case of other pediatric malignancies, treating patients with NRSTS is complex and demands a multimodal approach. The rarity and diversity of these tumors mean that children and adolescents with NRSTS need to be referred to selected institutions with adequate experience, and with multidisciplinary skills in enrolling patients in clinical trials.

Though there is no evidence to support any biological differences between the same STS occurring in children and adults, until recently an adolescent with an adult-type NRSTS and a young adult with the same tumor were treated very differently - and sometimes still are - particularly as concerns the use of chemotherapy. Cooperation needs to be improved between pediatric oncologists and adult medical oncologists dealing with very similar diseases, and there are now findings to suggest that this process is underway.

Oncologists have great expectations concerning the development of new agents for STS, but it is very important to emphasize that - while awaiting new systemic therapies - we must seek to make better use of the standard therapies already available. Though NRSTS (like adult STS) have to be considered globally as tumors of uncertain chemo sensitivity, there is no doubting that some subsets of patients benefit from chemotherapy.

References

Role of Chemotherapy in non-Rhabdomyosarcoma Soft Tissue Sarcoma

Milena Villarroel

Abstract
Non-Rhabdomyosarcoma Soft Tissue Sarcoma (NRSTS), are a rare, heterogeneous group of many different neoplasms of mesenchymal origin with variable biologic potential, whose clinical course and outcome have not been well defined. Although surgery and radiotherapy are known to be effective for the local treatment of soft tissue sarcoma, the role of chemotherapy in the management of patients with NRSTS remains controversial. Adjuvant chemotherapy regimens containing doxorubicin alone or in combination with ifosfamide have been the most widely used strategy, with a reduction in the risk of local relapse and in the risk of distant relapse, but this benefit has not translate into overall survival for those receiving chemotherapy. Evidence also suggests that chemotherapy would benefit specially certain groups of patients as those with extremity, high-grade, large size tumors. Synovial sarcoma, the most common pediatric NRSTS, is an entity whose response to chemotherapy is considered halfway between the most typical adult soft tissue sarcoma and the chemosensitivity of pediatric rhabdomyosarcoma. The analysis of these features may be helpful in selecting patients for including chemotherapy in a multimodality approach, thus designing future tailored trials.

New chemotherapy regimens according to histology are being tested. Molecular rearrangements may also serve as targets for designing specific therapies with the fusion gene product. The use of signal transduction inhibitors or angiogenesis inhibitors, could complement therapies for long-term control of disease.

The rarity of these tumors makes necessary the conduction of prospective multinational collaborative trials that allow answering all these relevant questions.

Introduction
Soft Tissue Sarcomas (STS) account for a fraction of 1% of all cancer diagnoses in all ages, but representing approximately 7.4% of all cancers in patients younger than 20 years. Rhabdomyosarcoma (RMS) comprises approximately 40% of the pediatric STS, and has been extensively studied, while the remaining diseases, Non-Rhabdomyosarcoma Soft Tissue Sarcoma (NRSTS), are a rare, heterogeneous group of many different neoplasms of mesenchymal origin with variable biologic potential, whose clinical course and outcome have not been well defined. These characteristics have precluded collaborative and prospective clinical trials that would provide guidelines for a logical treatment strategy.

The optimal management and treatment strategies are related to the primary anatomic site and extent of both local and systemic disease. Although surgery and radiotherapy are known to be effective for the local treatment of STS, the role of chemotherapy in the management of pediatric patients with NRSTS remains controversial. Successful treatment requires a coordinated multidisciplinary approach that includes the pediatric surgeon, radiation oncologist, and pediatric oncologist.

Because NRSTS are more numerous in adults, there is more information available about their natural history and treatment; and most of the information regarding chemotherapy is drawn from adult published data. Although the general approach to children with these tumors is often similar to that for adults, there are important differences, as the biology of the childhood NRSTS may differ significantly from that of the adult counterpart. On the other hand most of the experience gained in the treatment of pediatric NRSTS has derived from the management of RMS.
Chemotherapy Regimens

Active agents in adjuvant setting

Adjuvant chemotherapy trials in adults have important limitations. Most of these studies are small, inadequately powered to identify small improvements in outcome, and include relatively heterogeneous patient populations making their interpretation more difficult.

Doxorubicin

A meta-analysis published in 1997, of 14 randomized trials of adjuvant chemotherapy containing regimens with doxorubicin (alone or in combination) comprising 1,568 adult patients with NRSTS was conducted. Patients were older than 15 years, had localized disease, had not experienced local recurrence nor had received induction chemotherapy. This study demonstrated a 27% reduction in the risk of local relapse and 30% reduction in the risk of distant relapse at 10 years, but this did not translate into overall survival (OS) benefit (11% reduction in risk of death, \( p = .12 \)) for those receiving adjuvant chemotherapy with doxorubicin-based regimens. There was no evidence that the result was influenced by whether the trials used doxorubicin alone or in combination with other drugs. The results were similar in an analysis of death from soft-tissue sarcoma, with potential absolute benefit of 4% (95% CI 1–9) at 10 years, representing a possible survival improvement from 50% to 54%.

Regarding subgroup analysis, fewer data were available for histology, grade, and tumour size. For overall survival, there was no clear evidence to suggest that any subgroup benefited more or less from adjuvant chemotherapy. Of interest, among patients with lesions of the extremities the absolute benefit at 10 years was 7%, showing the clearest evidence of a treatment effect on survival. Despite limitations it appeared that chemotherapy carried some benefit, at least for certain patients with localized NRSTS. (1)

A randomized clinical trial performed by the Italian Sarcoma Group tested the efficacy of a combination of ifosfamide and epirubicin in 104 patients with high-grade, large, primary extremity NRSTS (including recurrent cases). Patients between 18 and 65 years of age with high-grade sarcomas (primary diameter 5 cm or any size recurrent tumor) in extremities or girdles were eligible. Stratification was by primary versus recurrent tumors and by tumor diameter greater than or equal to 10 cm or less than 10 cm. After a median follow-up of 59 months, 60 patients had relapsed (28 in the treatment arm and 32 in...
the control arm) and 48 died (20 in the treatment arm and 28 in the control arm). The median disease-free survival (DFS) was 48 months in the treatment group and 16 months in the control group \( (P = .04) \); and the median OS was 75 months for treated and 46 months for untreated patients \( (P = .03) \). For OS, the absolute benefit deriving from chemotherapy was 13% at 2 years and increased to 19% at 4 years \( (P = .04) \). It was concluded that intensified adjuvant chemotherapy with addition of ifosfamide, had a positive impact on the disease-free survival (DFS) and OS of patients with high-risk extremity soft tissue sarcomas, and although cure was still difficult to achieve, a significant delay in death was obtained with short duration of treatment and absence of toxic deaths. (4)

On the other hand, the interim analysis of a phase III European Organization for Research and Treatment of Cancer (EORTC) Soft Tissue & Bone Sarcoma Group (STBSG) multicentre randomised trial was recently reported. The purpose was to determine the impact of intensive adjuvant chemotherapy on survival for excised high grade STS. Between 1995 and 2003, 351 patients were recruited and randomised to observation or chemotherapy with doxorubicin and ifosfamide. An interim analysis for futility has been performed, because survival in the observation arm was better than expected: estimated 5-yr recurrence-free survival was 52\% in both arms; OS 69\% (observation arm) and 64\% (chemotherapy arm), failing to show a survival advantage for adjuvant chemotherapy. (5)

There was a recent communication of an exploratory, retrospective analysis performed on the EORTC STBSG database. Data on 1,319 chemotherapy-naïve patients receiving ifosfamide for advanced STS were analyzed. Among analyzed factors were gender, age, histologic type, histologic grade, disease sites, and type of ifosfamide treatment (single vs. with doxorubicin) and ifosfamide dose \(< \text{ and } \geq 9 \text{ gr/m}^2 \text{ per cycle})\). Combination with doxorubicin, high grade and histologic type (Synovial Sarcoma vs. Leiomyosarcoma) were associated with improved response rate. Compared to doxorubicin single agent, predictive factor analyses revealed that patients who benefited less from ifosfamide were some STS entities (Leyomiosarcoma and liposarcoma) and patients without liver metastases. Thus, although studies do not show a survival advantage for adjuvant chemotherapy, this retrospective analysis may be helpful in selecting patients for ifosfamide, and in designing future tailored trials. (6)

**Pediatric Studies**

Translating the data from adult STS studies into recommendations for the treatment of pediatric patients is not correct because few studies in adults have utilized dose-intensive regimens that would be well tolerated in pediatric patients, so response rates and outcomes might be superior in pediatric populations treated with higher-dose regimens compared to those reported in adults.

The only published randomized pediatric adjuvant chemotherapy study, conducted by the Pediatric Oncology Group between 1986 and 1992, was a randomized comparison of VACA (vincristine, actinomycin D, cyclophosphamide, and doxorubicin) to observation alone. Only 30 of the 81 eligible patients accepted randomization and among the patients who accepted randomization, event-free survival (EFS) and OS were inferior in the group that received chemotherapy, the same as the findings in the group as a whole. In both arms there was an imbalance in patients with high-grade tumors, with a greater proportion of patients with high-grade tumors assigned to the chemotherapy arm. The differences in outcomes disappeared when the analyses of EFS and OS were stratified by tumor grade. Event-free survival was found to be significantly worse in patients with high grade tumors, regardless of their randomization status. This clinical trial did not adequately determine whether or not adjuvant chemotherapy is beneficial in pediatric patients with grossly resected STS, but confirmed that high histologic grade is a major determinant of the risk of developing metastases. (7)

The Italian and German pediatric groups reviewed their databases to evaluate whether adjuvant chemotherapy can be recommended for high-risk, surgically-resected, adult-type non-rhabdomyosarcoma soft tissue sarcomas within the new European Pediatric Soft Tissue Sarcoma Study Group (EpSSG) protocol. Patients classified as group I-II, with high-grade tumor (G3) larger than 5 cm in size, were analyzed. Thirty-six patients were included and the clinical features
and outcome of the group of 21 patients who received chemotherapy were compared to the group of 15 patients treated with local treatment only. For the series as a whole, 5-year EFS was 26.2%, metastasis-free survival (MFS) 34.0%, and OS 37.5%. In patients treated with chemotherapy, MFS was 49.5% and OS 41.5% (median time to relapse: 13 months). In patients who did not receive chemotherapy, MFS was 0% and OS 23.8% (median time to relapse: 3 months). The role of adjuvant chemotherapy in NRSTS is still uncertain, however, this retrospective analysis showed that patients with high grade and large-size tumors have a high-risk of metastatic spread, and that MFS appears to be better in patients who received chemotherapy. (8)

Other Chemotherapy Strategies: Neoadjuvant Chemotherapy

Pediatric clinical trials that have evaluated neoadjuvant chemotherapy for the treatment of unresectable or metastatic NRSTS, included a randomized study conducted between 1986 and 1994. Seventy-five patients comparing VACA (vincristine, actinomycin D, cyclophosphamide, doxorubicin) to VACAD (VACA plus dacarbazine). Sixty-one patients were considered eligible and received chemotherapy and radiation therapy to the primary tumor and areas of metastases; 36 patients had regional unresected (Group III) disease, and 25 had metastatic disease (Group IV) at time of enrollment. Fifty patients were randomized (25 to each treatment arm) and the remaining 11 patients were non-randomly assigned to VACA due to a shortage of dacarbazine. Only 28 (46%) experienced a complete or partial response to neoadjuvant chemotherapy. (9)

A second multicentric pediatric clinical trial conducted between September 1996 and June 2000 described response rate and survival of 39 children and adolescents with unresected or metastatic NRSTS treated with vincristine, ifosfamide, and doxorubicin plus granulocyte colony-stimulating factor. The estimated 3-year OS rate was 59% ± 8.2% and progression-free survival (PFS) for eligible patients was 43.6% ± 7%. Patients with clinical group III disease had significantly better 3-year OS and PFS rates compared with patients who presented with metastatic disease. This regimen was moderately active against pediatric NRSTS. Patients with synovial sarcoma had higher response rates, and patients with unresected disease had improved outcomes. Patients with metastatic disease had a poor survival. (10)

The published experience in adults with soft tissue sarcomas treated with neoadjuvant chemotherapy is similar to that of childhood NRSTS, it is well-tolerated and appears to have no significant impact on postoperative morbidity. Rates of response range from about 30% to 50%, and dose-intensive regimens may induce objective responses in up to two-thirds of patients. Although tumor responses are documented, it is unclear what proportion of those patients with initially unresectable primary tumors are able to undergo gross tumor resection following induction chemotherapy. Furthermore, up to 20% of patients experience tumor progression following neoadjuvant chemotherapy. Thus, outside of a clinical trial setting, neoadjuvant chemotherapy should be considered only for children with unresectable or metastatic NRSTS (11).

Dose-intensification

The poor outcome of patients with high-risk NRSTS has led to the evaluation of chemotherapy dose escalation as a strategy to improve outcome. A number of phase I and II clinical trials in adults have shown that dose escalation of known active agents can modestly improve response rates, providing the rationale for studying more intensive chemotherapy with hematopoietic stem cell rescue. Very few small size, not randomized studies have been conducted to date.

For pediatric NRSTS there have been no studies evaluating high-dose chemotherapy with stem cell support.

For the time being, dose-intensive chemotherapy with stem cell rescue must be considered investigational. (12)

Prognostic Factors

Clinical Features

In a retrospective analysis of children and adolescents with initially resected NRSTS treated at St Jude Children’s Research Hospital (SJCRH), high histologic grade, large tumor size,
invasiveness, and positive microscopic margins were unfavorable prognostic features for event-free survival (EFS) and overall survival. (13)

In initially unresected nonmetastatic NRSTS seen at the same institution between 1962 and 1996, the presenting clinical features and tumor characteristics of 40 pediatric patients, were retrospectively reviewed. More than 70% of these patients had tumors with high-risk features (tumor size > 5 cm, high grade, invasiveness). Twenty-seven patients for whom complete treatment information was available, was analyzed to determine whether response to therapy was associated with local disease control, EFS and OS. Five-year EFS estimate was 33% +/- 9%?and OS 56% +/- 10%. Ten (37%) of these patients had a complete or partial response to neoadjuvant chemotherapy and/or radiotherapy, and 2/10 had residual tumor after surgery. Combined chemotherapy and radiotherapy seemed more effective, but the response to neoadjuvant therapy did not predict outcome. Most treatment failures were local, and postrelapse survival was poor (19% +/- 10%). (14)

**Histologic types**

When staging pediatric NRSTS it is difficult to determine which grading system to use, in order to account for the unique histologic subtypes that occur in childhood; for example, ?brosarcoma and hemangiopericytoma in very young children, that have a very favorable clinical outcome despite an aggressive histologic appearance. Pediatric Oncology Group (POG) developed and prospectively validated a pediatric NRSTS grading system that incorporates mitotic index, necrosis, cellularity, and nuclear pleomorphism for most tumors, while assigning certain histologic subtypes categorically to a grade, based on their clinical behavior. (12)

Given the heterogeneity of these tumors, clinical studies should target diagnostic subgroups as specifically as possible. The rarity of each histology subset prevents the performance of clinical trials on single tumor types, and consequently, NRSTS have to be analyzed as a group. At the Istituto Nazionale Tumori (INT) in Milan an effort was done to create a more homogeneous group of pediatric NRSTS to analyze. Focus was placed on NRSTS that are typical of adulthood (excluding infantile ?brosarcoma), definitely malignant (excluding borderline tumors, ie, hemangioendothelioma) and that have morphologic features resembling differentiated/mature tissues (excluding small round-cell tumors, ie, RMS, Ewing's Sarcoma Family of Tumors, and desmoplastic small round-cell tumor). These tumors were defined as adult-type soft tissue sarcomas. A retrospective analysis of 182 consecutive patients with these characteristics seen at INT, in a 25-year period was reported. The subset of adult-type sarcomas arising in a pediatric population is comparable to reported series on STS in adult age, with most frequent localization at extremities.

Findings confirmed synovial sarcoma (SS) and Malignant Peripheral Nerve Sheath Tumor (MPNST) as the most frequent diagnoses in adolescents and young adults.

The prognostic factors of adult sarcomas (ie, large tumor size, local invasiveness, high-grade tumors, older age, and sites other than the extremities) remained their value in this series, but the proportion of pediatric patients with unfavorable features seems quite lower than in adults (ie, tumors larger than 5 cm and high-grade tumors were present in approximately one half of the patients versus two thirds of patients in adult series). (15)

Synovial sarcoma is the most common childhood NRSTS. The most common site of metastasis is the lung; however, unlike most other NRSTS, SS can also spread to regional lymph nodes. Controversy still exists about prognostic factors and whether adjuvant chemotherapy is useful in treating these patients. A retrospective, multicenter multivariate analysis in 219 children and adolescents diagnosed with SS, was conducted to identify prognostic factors related to outcome. The median age at diagnosis was 13 years, and the median follow-up was 6.6 years. The estimated 5-year OS was 80% +/- 3% and EFS 72% +/- 3%.

Intergroup Rhabdomyosarcoma Study (IRS) grouping, tumor invasiveness and tumor size, were statistically significantly related to outcome. A previously unreported interaction between tumor size and invasiveness was identified, and proved the most consistent statistically significant variable associated with poor outcome. Histologic
information on tumor grade should be incorporated into future trials to investigate its role in outcome in patients with SS.

The role of adjuvant chemotherapy in patients with SS has been controversial. Although responses have been reported with combinations of alkylating agents and anthracycline, the influence on survival remains debatable, especially in patients with grossly resected localized tumors.

A report from the German CWS group, concluded that there was a beneficial effect on outcome in patients who received chemotherapy, compared to historical controls. Distant recurrence rates of near 40% are much higher in the adult patients compared to 21% observed in this study, thus confirming that relative to adult patients, children and adolescents with SS have a better outcome. This difference is possibly due to more widespread use of chemotherapy in the pediatric population, larger tumor sizes in the adult patients, and unknown biologic differences between the two age groups. In this study, patients who were not treated with chemotherapy had similar outcomes to patients treated with chemotherapy whether metastatic patients were included in the analyses or not. (16)

Conclusions and Future Directions

Based on existing data of adults and children, for the time being, the role of adjuvant chemotherapy for surgically resected NRSTS has not been established, and is not considered standard of care. For the remaining patients with advanced disease, who do require treatment beyond surgery alone, the optimal application of modalities currently in use must be evaluated. Regarding chemotherapy, currently a full-dose doxorubicin and ifosfamide regimen is of choice. The effective use chemotherapy and radiotherapy to achieve gross tumor resection in patients with unresectable disease is another issue to address in future research.

NRSTS are analyzed as a group despite of their heterogeneous histology, so the selection of specific meaningful subgroups, i.e. adult-type soft tissue sarcoma and SS, is very important. New chemotherapy regimens according to histology are being tested, i.e. gemcitabine and docetaxel in patients with metastatic or relapsed leiomyosarcoma, paclitaxel in angiosarcoma, imatinib in dermatofibrosarcoma protuberans, and ET-743 in myxoid liposarcoma.

Cytogenetics and molecular analysis of fusion or mutated genes is used in diagnosis, prognosis, and design of biological treatments i.e treatment of patients with gastrointestinal stromal tumours expressing mutant c-kit with a specific tyrosine kinase inhibitor, STI571. Molecular rearrangements may also serve as targets for designing specific immunotherapies with the fusion gene product. The use of signal transduction inhibitors or angiogenesis inhibitors, could complement existing treatments for long-term control of disease.

The rarity of these tumors makes necessary the conduction of prospective multinational collaborative trials that allow answering all these relevant questions.

References


Surgery of non-rhabdomyosarcoma soft tissue sarcoma

Samuel Aguiar Junior

Abstract
Almost half of the childhood soft tissue tumors are classified as non-rhabdomyosarcomas (NRSTS). As the chemosensitive of NRSTS are quite unpredictable, surgery has an important role in the treatment of these tumors. A wide resection with three-dimensional negative margins is the treatment of choice, in localized disease, and the resecability must be well planned by the surgeon before treatment. When the size and location preclude the achievement of negative margins, mutilations must be avoided, as systemic relapses cannot be prevented only with radical surgeries. In these situations, marginal and functional sparing procedures are recommended, in association with adjuvant or neoadjuvant radiation therapy, for local control. The toxicity of radiotherapy in childhood stimulates the investigation of the use of chemotherapy as a neoadjuvant strategy for improving functional sparing procedures and local control, especially in some subtypes of tumors which appears to be more chemo-sensitive, as synovial sarcomas.

Introduction
Soft-tissue sarcomas are malignant neoplasias of mesenchymal origin account for about 8% of childhood cancers. Little more than half of these tumors are classified histologically as rhabdomyosarcomas (RMS) and the other 40% to 50% are part of an heterogeneous group of tumors classified as non-rhabdomyosarcomas (NRSTS). Apart from histological differences between these two great sub-groups, there are also therapeutic and prognostic implications.

The well-known chemosensitivity of rhabdomyosarcomas makes surgery part of a multidisciplinary approach where chemotherapy plays a primordial role. In addition, non-rhabdomyosarcomas have a behavior similar to that of adult sarcomas, where low response rates to chemotherapy are observed, except for the neuroectodermal origin tumors. This places surgical resection as the main, and sometimes the only, therapeutic approach for non-rhabdomyosarcomas.

Classical surgical concepts
Histological diagnosis of soft-tissue sarcomas requires a biopsy procedure, which can be open or a percutaneous core (Tru-Cut) biopsy. Open biopsies can be incisional, when only a sample of tumor tissue is removed for analysis, or excisional, when the entire tumor is removed and submitted to a histological diagnosis. Excisional biopsies are not recommended due to compromising a subsequent adequate resection and are carried through only in rare case of small superficial tumors. Although simple, biopsy procedures must follow the basic principles of asepsis, hemostasis and incision planning. A hematoma or infection caused by a biopsy may have disastrous consequences in subsequent therapeutic planning. Moreover, one must remove the biopsy scar when performing surgery. Therefore, the incision for a biopsy must be done in the area where one plans surgery incision for tumor resection and along the member longitudinal axis for making en bloc resection easier.

Curative intent surgery requires a wide three-dimensional resection with adequate margins of at least 1,0 cm of surrounding normal tissue (Figure 1). Pre-treatment evaluation must predict adequate or non-adequate margins through a surgical planning that takes into account a physical exam associated to a high-quality image exam (CT scan or magnetic resonance). In cases of children presented with previous inadequate unplanned resection, a wide reexcision must be performed, whenever possible. This is justified by the high rates of microscopic residual disease, which can be as high as 48%.
It is common that the size and mainly the site of the tumor do not allow obtaining adequate margins. This is particularly problematic in tumors of the retroperitonium, head and neck, and in deep tumors of extremities. As usually happens in the treatment of adult sarcomas, the proximity of lesions to neurovascular or bone structures determined high rates of amputations during some decades in order to get adequate margins. Despite the radical treatment, most patients died due to recurrence in distant organs, particularly in the lungs. Results published from the 1980’s by institutions that performed conservative surgeries associated to adjuvant radiotherapy showed good rates of local control, from 78% to 91% without changes in global survival rates.\textsuperscript{7,8} Two prospective and randomized studies validated these results.\textsuperscript{9,10} Currently, amputations and disarticulations are carried through in only 5% to 10% of cases.\textsuperscript{2}

On the basis of the history of treatment of adult extremities sarcomas, mutilating surgeries for obtaining microscopic negative margins are not recommended for childhood non-rhabdomyosarcomas. For tumors in unfavorable location, where pre-treatment locoregional staging predicts difficulties for attaining negative margins, marginal resections (Figure 2) with functional preservation (adjacent to tumor pseudocapsules, but without breaking it)\textsuperscript{4}, associated to adjuvant radiotherapy, are recommended.

\textbf{Figure 1:} Example of wide resection of a NRSTS located at lateral compartment of the thigh. Tumor is completely surrounded by at least 1.0 cm of normal tissue.

\textbf{Figure 2:} Example of marginal resection of a tumor adjacent to the femur. Dissection was made between the periosteum and the tumor pseudocapsule, with limb sparing.
Low Grade Tumors

Histological low-grade sarcomas, such as fibrosarcomas and hemangiopericytomas, as well as desmoid tumors and neurofibromas, are treated by surgery alone, and complete surgical resection is the first-choice treatment, whenever feasible. When resection implies in important esthetic or functional loss, surgery may be delayed, as these tumors have a low metastatic potential. The moment of surgery depends on the time of progression and the functional viability of the affected member. For instance, an amputation due to a desmoid tumor can only be considered when the affected member loses its function because of the disease local progression. In these situations, there are reports of other modalities of treatment, such as chemotherapy, radiotherapy or hormonal blockade, but their results are very unpredictable. Particularly for infantile fibrosarcomas, there are also reports of surprising responses to neoadjuvant chemotherapy, a procedure which prevents mutilations.\(^{11,12}\)

High grade tumors

Wide or radical resection is the main modality for locoregional control of high-grade sarcomas. For non-metastatic tumors and which ample and negative margins can be achieved, surgery is the only modality of treatment. But these tumors have a high metastatic potential and local radicality does not modify the natural history of the disease regarding systemic recurrence.\(^{2,13,14}\) Due to this, for high-grade NRSTS sarcomas where resection with adequate margins would imply a serious functional or esthetic loss, a marginal conservative resection, associated with radiotherapy, is the recommended strategy for local control.

The modality (external radiotherapy or brachitherapy) and the moment for using radiotherapy (pre- or postoperatively) is still controversial. The initial approach with surgery has the advantage of lower rates of complications of the operative wound. Some advantages are pointed out in favor of preoperative radiotherapy: 1) a better tissue oxygenation without previous surgical manipulation, which may increase the cytotoxic effect of radiotherapy; 2) smaller and less normal tissue included when planning is preoperative; 3) as it reduces tumor volume, preoperative radiotherapy seems to make surgery technically more feasible when attaining adequate three-dimensional margins is not possible.\(^{15}\) The disadvantages of neoadjuvant radiotherapy relate mainly to the high rates of operative wound related complications.\(^{16,17}\)

Although there are patent benefits of radiotherapy for local control of sarcomas of extremities, one must consider toxicity in pediatric patients, mainly late toxicity.

Effects of adjuvant chemotherapy in non-rhabdomyosarcomas remain controversial both for adults and children.\(^{18-21}\) The use of neoadjuvant chemotherapy in locally advanced tumors was not much studied until now. In adults, the use of neoadjuvant systemic chemotherapy was tested in only one prospective and randomized trial, which showed an objective response rate of 29% for pre-operative chemotherapy with doxorubicin and ifosfamide.\(^{22}\) In children, an Italian publication analyzed 52 patients with non-metastatic tumors of extremities considered unresectable, all of them NRSTS, submitted to neoadjuvant chemotherapy. The study was retrospective and involved several chemotherapy protocols. The rate of total objective response was 34.6%. Thirty three tumors were resected after neoadjuvant treatment, 6 of them involving amputations.\(^{23}\)

A North American multi-institutional prospective phase II study (Pediatric Oncology Group) analyzed 39 patients under 21 years of age having unresectable or metastatic NRSTS regarding neoadjuvant chemotherapy with vincristine, ifosfamide and doxorubicin. The rate of objective response was 41%. Among 25 patients with locally unresectable and non-metastatic tumors, 17 were operated after induction chemotherapy, with 3 amputations.\(^{24}\)

In these two studies, the sub-group of synovial sarcomas had significant better response rates.
neuroectodermal tumors (PNET’s) or extraosseous Ewing family tumors have to be treated similarly to bone tumors and included in neoadjuvant chemotherapy protocols for Ewing osseous tumors. Surgical resection after neoadjuvant treatment follows the same principles of resection for all other soft tissue sarcomas. 25

Lymph node approach

In contrast to rhabdomyosarcomas, NRSTS have low rates of lymph nodal metastases similar to adult sarcomas. This way, lymph node dissection is only justified for non-rhabdomyosarcoma when there is obvious lymph nodal compromising. Some histological subtypes have higher rates of lymph nodal metastasis, such as epithelioid sarcomas, clear-cell sarcomas, alveolar soft part sarcomas, and angiosarcomas. In these rare situations, sentinel lymph node biopsy may be considered. 26

Summary

Wide resections with clear negative three-dimensional margins are the primordial treatment of non-metastatic NRSTS. Resecability must be well planned before treatment by the surgeon physical exam in association with CT or MRI. In cases of previous inadequate unplanned resection, a reexcision must be performed for obtaining negative margins. However, the size and the location of the tumors commonly preclude a wide resection. In these cases, mutilating procedures are not recommended, as systemic relapses are not prevented. So, marginal resections with functional sparing must be performed, whenever possible, in association with multidisciplinary adjuvant or neoadjuvant treatment.

References


Radiotherapy in the management of Pediatric Non-Rhabo Soft Tissue Sarcoma

Valerie Bernier

Abstract
Radiotherapy is necessary to obtain local control, in association with surgery and chemotherapy, in most of the case of Non Rhabdo Soft Tissue Sarcoma. Indications are validated for high risk of local relapses, so for IRS II and III patients, and for high grade tumors. Radiation could be avoided for low grade lesions less than 5 cm and IRS I. The indication for children younger than 2 years could be modulate, due to the great proportion of low grade tumors and low growth rate, even in case of positive resection margins. Minimizing toxicity by the use of techniques sparing normal tissue is recommended, in a multidisciplinary approach in experimented team, including brachytherapy, IMRT, tomotherapy... Caution must be taken for long term morbidity, especially for those new techniques, which require assessments using clinical trials.

Introduction
Approximately 8 % of childhood cancers are soft tissue sarcomas (STS), with rhabdomyosarcoma representing approximately 60 % of them. The so-called “non-rhabdomyosarcoma soft tissue sarcomas” (NRSTS) account for about 3-4% of pediatric cancers. This entity constitutes a very heterogeneous group of tumors with a variety of histotypes with different origins, biology and natural history, arising in any part of the body with a majority of extremities localization, and preponderance of synovialosarcomas, especially in older patients.

Most of treatment strategies for pediatric NRSTS derives from the experience of managing the same diseases in adults or is based on the principles derived from the management of RMS. However, prognostic factors in pediatric NRSTS are not completely defined and it is uncertain whether they are the same of those identified in adult sarcomas.

Pediatric NRSTS have usually been staged according to the IRS system, with some differences from the adults system. Prognostic factors associated with increased risk of local relapse include size tumor > 5 cm, deep location, lack of radiotherapy use, and microscopically positive margins. Prognostic factors associated with increased risk of distant recurrence included tumor size, nodal status, invasive tumor and high histologic grade. Resectability, depending also on location and size tumor, is a very important and independent factor, predicting both local and distal relapses. More recently, age appears to be an independent and important factor. Their propensity to metastasize is directly correlated to their grade of malignancy. Usually, they are characterized by local aggressiveness, emphasizing the important role of local control. Indications and procedures of radiotherapy (RT) are very difficult to standardized, due to the great variability of histology, tumor locations, extent of surgery and age of patients. The rarity of NRSTS contributes to the lack of pediatric trial data.

The first therapeutic question is how to best obtain local control, with less morbidity, in a multidisciplinary approach with surgery, radiotherapy and chemotherapy.

Generally, low-grade tumors may have local aggressiveness but low tendency to metastatic spread. High-grade tumors are more frequent and have a more invasive behaviour with high propensity to metastasize (in particular at the lung).

Overall, the survival rate for soft tissue sarcomas averages 60%, with substantial differences according to the different histotypes, the degree of malignancy, and the stage of the disease. Surgery is the mainstay of treatment in NRSTS, however, the role of surgical extent on the outcome remains to be defined for each histology subgroup.
With the exception of pPNET/Ewing sarcomas (and partially of synovial sarcomas), NRSTS are generally considered poorly chemosensitive tumors. However, knowledge regarding chemotherapy responsiveness is clearly incomplete and must be improved.

Integrating the radiation oncologist in a multidisciplinary approach

The radiation oncologist - with experience in pediatric radiotherapy - must be involved early in the discussion of therapeutic management, to appreciate the morbidity of potential radiation, and the possibilities of special techniques sparing organs at risk (Intensity modulation RT (IMRT), brachytherapy, expanders, clips,...). Discussions with radiologists and surgeons are very important to appreciate the target volume, before and after any surgical act.

Problems related to radiotherapy

Age

Radiotherapy induced late sequelae, all the more important since the child is younger. The median age of RMS and NRSTS is around 5 years.

Paulino et al (3) report the good prognosis for children younger than 2 years, probably due to the great proportion of low grade lesions, and suggest to avoid RT for this young population, even in case of marginal resection.

Radiation-sensitive genetically susceptible pediatric subpopulations

Li-Fraumeni syndrome and above all Neurofibromatosis type I are at higher risk of developing soft tissue sarcomas, particularly malignant peripheral nerve sheath tumors (MPNST) which has also been reported as a second malignant neoplasm (4, 5).

Their features are more often embryonal, pelvic or genitourinary tract locations and onset on early age. The outcome of sarcoma is poorer in case of NF1 due to the more aggressive clinical characteristics, and less chemosensitivity (6-8).

Moreover, progressive irradiation-induced occlusive vasculopathy may occur, predominantly in NF1 patients (8, 10). It has been most frequently reported after intracranial irradiation (11). Cerebral vasculopathy has been observed after doses as low as 25 Gy. Several types of radiation procedures have been associated with these irradiation-induced vascular changes, however, the potential relation between radiation strategies used and progressive vascular changes cannot be stated, since in most reports detailed descriptions of irradiation techniques, field size, fractionation, and total dose are lacking.

Differences between children and adults

NRSTS occur at any age, but some subtypes are more age-specific which increases the complexity of outcome analysis. For example, synovial sarcoma, MPNST and fibrosarcoma (liposarcoma and malignant fibrous histiocytoma the most common histotypes in adult age) are more frequent in adolescents. Usually, they are characterized by local aggressiveness and their propensity to metastasize. In addition, prognostic factors in pediatric NRSTS are not completely defined because it is uncertain whether they are the same of those identified in adult sarcomas, and most treatments for pediatric NRSTS derives from the experience of managing the same diseases in adults, three Cooperative Groups (SIOP MMT (International Society of Paediatric Oncology – Malignant Mesenchymal Tumours) Committee - CWS (German Co-operative Soft Tissues Sarcoma Group) Committee - AIEOP STSC (Associazione Italiana Ematologia Oncologia Pediatrica - Soft Tissue Sarcoma Committee has led to the foundation of the European paediatric Soft Tissue Sarcoma Study Group (EpSSG) and designed a protocol (EpSSG NRSTS 2005), specifically for Localized Non-Rhabdomyosarcoma Soft Tissue Sarcomas.

Technical aspects

As for any pediatric irradiation, immobility must be obtained, by personalized system (customized molds, mask, sometimes general anesthesia). 3D dosimetry must be done on CT scan.

Target Volume encompassed the pre-operative tumor volume, visualized on MRI, adapted to the post-operative cavity, added with a 3 cm margin (usually less in case of brachytherapy), it is important not to irradiate all the circumference of a limb; scar and drain must be included, specially if the surgery report is unclear. But the total volume of irradiation should be reasonably adapted to location and age.
Sequence: pre or post operative RT
The advantages of pre-operative RT are to improve resectability, to decrease the possibility of intraoperative contamination and sometimes to allow limb preservation; some studies reported excellent results in high grade tumours with local control rate of 83-95 % and 5-year disease free survival rates of 56-74 % (12-14). The main problem is wound complications, with a rate of 6 to 30 % (15). An association with intra or post-operative RT is often necessary. No pediatric data comparing pre and post operative RT are available; in adults, the NCIC phase III trial reported, for NRSTS of the extremities, a greater rate of wound complications for pre-operative group (35 versus 17 % in post-operative), but an overall survival advantage in the pre-operative group, due to a smaller treatment-related or non tumor related deaths (16).

The advantages of post operative RT are the possibility of immediate surgery without delay for wound healing, and the obtention of non modified histopathologic analyses. In contrast, the volume of post-operative RT included scar is larger than pre-operative RT. In high grade tumors, local control rate is about 78-92 % and 5-year disease free survival rates 60-68 %. The impact of time between surgery and RT is not clearly defined (17).

Dose and Fractionation
Dose for pre-operative RT is 45 to 50 Gy. Several studies (1, 3, 18) used a post operative dose from 55 to 65 Gy with conventional fractionation (1.8 to 2 Gy/day), depending on the grade and the margin resection. The CWS studies 86 and 91 used a Hyper fractionated Accelerated RT (HART) with 2 x 1.6 Gy/d. The total dose was 32 Gy for favourable groups and 48 Gy for unfavourable groups. Results are similar to that achieved with conventional fractionation, mainly for RMS with a 5 year local control of about 44% (RT-) vs 63% (RT+) for high grade and negative margin, 43% (RT-) vs 82% (RT+) for low grade and positive margin, and 16 % (RT-) vs 55% (RT+) for high grade and positive margin. In the CWS 91 study, outcome seems improved, by intensifying chemotherapy for high grade tumours, with a 5-year EFS rate of 84% for synovialosarcomas, which compared favourably with published data (19, 20). In the retrospectively-analysed single-institution series from the Instituto Nazionale Tumori of Milan, out of 182 patients aged less than 18 years treated between 1977 and 2003 (synovial sarcoma 32%, MPNST 17%), 73 patients were treated with radiotherapy. Post-operative radiotherapy seemed to have an impact on local control and outcome in IRS group I patients considered at high-risk of local control due to large tumour size and in IRS group II patients. But the comparison with historical series must be carefully interpreted, due to progress in the same time of other techniques (radiology, surgery, ...). Thus, longer follow-up is necessary to compare late sequelae.

Specific techniques (brachytherapy, IMRT, tomotherapy,..)
Brachytherapy is certainly the most conformal radiation, with best tissue-sparing. The most common technique uses catheters placed during surgery, and loaded few days after. High dose rate sources present the advantage of pulse and large fractional doses, with some period without radiation and total time shorter than for low dose rate sources. The efficacy seems to be equivalent but long term morbidity has never been compared (21-23). The prospective randomized trial performed at the Memorial Sloan Kettering Cancer Center confirmed the benefit of brachytherapy on local control, especially in the high-grade lesions (24). Brachytherapy necessitates an experimented team, both for indication and technique, but must be integrated in the therapeutic strategy.

Recently developed, IMRT or tomotherapy offer the advantage of better conformal volume, decreasing the dose at organ at risk, especially for sites as head and neck (25). But beside the dosimetric benefit, and the eventual possibility to increase the dose in tumor volume for a better therapeutic ratio, the long term morbidity of those new techniques are totally unknown, and requires prospective evaluation. This technical RT progress is also associated to surgical and radiological progress, which contribute to a better therapeutic ratio. Further comparison with historical data should be cautious, and inclusion in clinical trial have to be encouraged.

Indications
In contrast to adults, there were only few pediatric
series of NRSTS; The largest single institution study from the St Jude Children’s research Hospital reported that a higher local relapse rate was observed if no radiation is performed after surgery, but on subset analysis, the local control was not improved using RT in case of negative margins of resection \(^{(1)}\).

Paulino et al \(^{(3)}\) reported a 5-year local control rate at of 100 % for group I disease (low grade, negative margins) without RT if tumor size is < 5 cm, and 63 % and 44 % for other group I patients, with or without RT respectively. Dantonello et al \(^{(26)}\) recently reported the results of the cooperative trial CWS 91: for the group A (IRSG I except unfavourable sites: extremities and head and neck), no radiation was performed, and results are similar compared with previous the CWS 86, with just change in chemotherapy, especially for favourable histology. But results for NRSTS are difficult to evaluate as a subgroup, due to the small number of patients.

The indication of RT is validated for all histological grade with positive margins after resection, except may be for children under 2 years and low grade tumors. Local control is improved by RT in several series \(^{(1, 3, 18)}\) with a 5 year local control of 82 % versus 43 % without RT. Overall survival is also improved by RT, due to the best local control. Only one third of children are surviving after a local relapse, and about 40 % of them subsequently developed distant metastasis and died \(^{(3)}\). Dantonello et al \(^{(26)}\) did not find a difference in OS between RT or no RT groups, in spite of a better local control with RT, but the profile risks of RT group were more unfavourable.

Location and histopathology are also important criteria in the indication for RT \(^{(27, 28)}\). In high grade soft tissue sarcomas, the local control rate is lower in upper extremity site and for positive resection margins. But the wound complication rate after surgery and radiotherapy is lower than in other sites, which suggests the possibility to increase the RT dose \(^{(29)}\).

The retrospective multicenter study coordinated by the MD Anderson cancer Center (MDACC) showed an increased local control rate with RT especially for synovialosarcoma \(^{(19)}\).

### Late effects
Late effects of RT are dose (total and by fraction), age and volume dependant. They occurred in about 13 % of the cases; musculoskeletal toxicities were the most common, limited overall activity levels, with decreased bone growth, soft tissue hypoplasia, osteonecrosis; peripheral nerve injuries are uncommon and occur at dose > 60 Gy \(^{(3, 30)}\); this toxicity is also added to the CT toxicity, leading to a quality of life deterioration, which can jeopardize the quality of life.

Special attention must be paid with new techniques, as IMRT or tomotherapy, in regards to late effects. The Childhood Cancer Survivor Study recently confirmed the increased second neoplasm with longer follow-up. Thyroid cancer seemed to be associated with radiotherapy doses less than 29 Gy \(^{(31)}\). It is necessary to pay attention with “low dose bath” from new techniques, even if Schneider et al \(^{(32)}\), in a theoretical study for genitourinary cancer did not find a increased risk of cancer with IMRT or protons.

### Summary
In conclusion, radiotherapy is necessary to obtain local control, in association with surgery and chemotherapy, in most of the case of Non Rhabdo Soft Tissue Sarcoma, and in a multidisciplinary approach. Indications and procedures of radiotherapy are not totally standardized, due to the great variability of histology, tumor locations, extent of surgery and age of patients. They are validated for high risk of local relapses, so for IRS II and III patients, and for high grade tumors. Radiation could be avoided for low grade lesions less than 5 cm and IRS I. The indication for children younger than 2 years could be modulate, due to the great proportion of low grade tumors and low growth rate, even in case of positive resection margins. No pediatric data comparing pre and post operative RT are available.

Minimizing toxicity by the use of techniques sparing normal tissue is recommended, in experimented team, including brachytherapy, IMRT, tomotherapy. Caution must be taken for long term morbidity, especially for those new techniques, which require assessments using clinical trials.
References


Pediatric Acute Myeloid Leukemia – Improving Survival One Patient at a Time

Raul C. Ribeiro, Ina Radtke, and Jeffrey E. Rubnitz

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Introduction

Progress in the treatment of pediatric acute myeloid leukemia (AML) has been relatively slow. Despite very intensive chemotherapy strategies, advanced supportive care, and ample access to a variety of hematopoietic stem cell transplantation (HSCT) procedures, the projected 10-year survival of patients diagnosed between 2005 and 2009 in the U.S. is only about 64% (95% CI, 54.1% to 72.6%)(1). Although this projected survival is at least 20% greater than that achieved in the early 1990s, it contrasts sharply with the 88% (95% CI, 85% to 90.5%) projected survival for acute lymphoblastic leukemia (ALL). Moreover, a substantial proportion of children treated for AML during the past 3 decades are expected to develop medical problems due to the treatment or its complications(2), particularly if they have undergone hematopoietic stem cell transplantation (HSCT)(3). Examining the strategies that have improved survival and the causes of mortality and morbidity may suggest new approaches to improve the survival and quality of life of children with AML and reduce the morbidity associated with treatment. In this presentation we review the clinical and biological features and outcome of pediatric AML and highlight the main challenges in moving the field forward.

The multifaceted acute myeloid leukemia: implications for prognosis and treatment

AML and related neoplasms are thought to result from clonal transformation of any of the myeloid cell precursors, which acquire constitutive self-renewing and proliferative properties. Although the mechanism of malignant transformation of myeloid precursors remains elusive, it is thought to involve the cooperation of multiple acquired genetic aberrations(4). The age adjusted- incidence rates of AML are approximately 1.8 per million individuals less than 20 years of age in the United States. Many children are diagnosed with AML during the first year of life; the incidence subsequently decreases, reaching a nadir at about 9 years of age, then slowly increases again until it reaches a second peak at approximately 75 years of age (http://seer.cancer.gov/index.html). These observations suggest that the mechanism of AML may differ across the age spectrum. Ethnic background and unidentified environmental factors appear to influence the incidence of AML and its subtypes. Children of Asian/Pacific Island origin appear to have an increased risk of AML(5), while acute promyelocytic leukemia (APL) is reportedly more frequent among individuals of Spanish, Italian, Mexican, and Central and South American ancestry(6). Granulocytic sarcoma is observed more frequently in Turkish(7), and Moroccan children (personal communication, Dr. Asmaa Qessar). However, because many of these regions lack population registry data, these reports are not confirmed.

Currently, acute myeloid leukemia (AML) is classified according to World Health Organization (WHO) criteria(8). In the most recent WHO classification scheme, the WHO Pathology Committee (in conjunction with a Clinical Advisory Committee, which includes pediatricians) has attempted to define homogeneous, biologically unique, and mutually exclusive entities on the basis of clinical context and the morphologic, cytochemical, immunophenotypic, and genetic features of the leukemic cells. In the previous WHO scheme, the AML subgroup “with recurrent genetic abnormalities” included AML with the
t(8;21)(q22;q22); RUNX1-RUNX1T, AML with the inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFA2-TMYH11, APL with the t(15;17)(q22;q12); PML-RARA, and AML with MLL rearrangements. The revised classification expands this subgroup to include new AML-specific chromosomal rearrangements and provides additional details about the existing ones. APL with other RARα translocations, such as those involving ZBTB16, NUMA1, NPM1 or STAT5B, is to be recorded as AML with the variant partner listed. The designation “AML with abnormalities of 11q23; MLL” has now been eliminated from the scheme, because different MLL abnormalities are associated with different clinical and prognostic correlates. For example, infant AML is commonly associated with MLL rearrangements, but the type of MLL rearrangement appears to have no prognostic significance(9). Conversely, older children with t(9;11)(p22;q23); MLLT3-MLL appear to fare significantly better than those with other MLL abnormalities(10). In addition, some very rare AML subtypes have been added to the classification, including AML with the t(6;9)(p23;q34); DEK-NUP214, AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1 and AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1. These subgroups are associated with unique clinical and biological features and therefore may provide insights into the mechanism of leukemogenesis and suggest potential therapeutic targets.

There is increasing evidence that not only acquired chromosomal abnormalities but also acquired gene sequence mutations and gene expression patterns have biologic, prognostic, and treatment implications. The best characterized gene abnormalities include sequence mutations of nucleophosmin member 1 (NPM1), CCAAT/enhancer binding protein α (CEBPA), neuroblastoma RAS viral oncogene homolog (NRAS), and stem cell factor receptor (KIT); internal tandem duplications (ITD) or point mutations of FMS-related tyrosine kinase 3 (FLT3); and partial tandem duplication (PTD) of the myeloid/lymphoid or mixed-lineage leukemia (MLL) gene. These gene mutations may occur in conjunction with AML-specific chromosomal translocation, in combination with each other, or in cytogenetically normal AML. When classified on the basis of their known physiologic properties and functional relevance, the mutations that activate signal-transduction pathways are designated class I mutations, whereas those that affect transcription factors or components of the transcriptional complex are class II mutations. Cooperation between class I and class II mutations has been postulated to generate the leukemic clone(11).

Although data relating these mutations to the outcome of AML are still evolving, some themes have emerged. FLT3 ITD mutations, which occur in about 30% of adult cases and 15% of pediatric cases of cytogenetically normal AML, have been consistently associated with poor prognosis(12). Conversely, NPM1 mutations, which are found in about 50% of adult and 25% of pediatric cytogenetically normal AML, are reported to be associated with a good prognosis(12). However, a patient may have a combination of leukemic-cell gene rearrangements and mutations, complicating the predictive analysis of outcome. For example, the presence of KIT mutations appears to negatively influence the otherwise favorable prognosis of patients with the t(8;21)(q22;q22); RUNX1-RUNX1T(13). Cases of APL with FLT3 ITD appear to have a poorer outcome than other APL cases(14). Similarly, in cytogenetically normal AML, the presence of FLT3 ITD cancels out the favorable implication of NPM1 mutation.

The prognostic implications of AML-specific genetic abnormalities are slower to emerge in pediatric AML, with its relatively small number of cases, lower frequency of gene mutations, complex pattern of combined genetic abnormalities (Figure 1), and different front-line and supportive treatments. There is no reason to believe that these data will be simpler than those in adult AML(15), and in fact they may be more complex if constitutional gene defects modify the predictive significance of the acquired abnormalities. The interactions between constitutional and acquired genetic findings are illustrated by pediatric acute megakaryoblastic leukemia(16, 17) (M7 AML; Table 1), which has a favorable prognosis in the context of constitutional trisomy 21 or acquired t(1;22)(p13;q13) RBM15-MKL1 fusion but a poor prognosis in cases with normal cytogenetics or acquired trisomy 21(18, 19, 20, 21). The first complete AML genome for a patient has now
been fully sequenced\(^2\)). As the technology develops, genome-wide approaches are being applied to AML to identify additional associated genetic lesions and investigate the relevance of aberrations like focal copy number alteration, loss of heterozygosity, and micro RNAs.

**Figure 1A:** Cytogenetic subtypes of childhood AML. Percentages reflect the range of these subtypes reported in several clinical trials. (adapted from Rubnitz JE, Razzouk BI, Ribeiro RC. Acute Myeloid Leukemia, in Childhood Leukemias Ed. Ching-Hon Pui, 2sd edition, Cambridge University Press, New York, pp 506, 2006, with permission).

**Figure 1B:** Overall frequency of sequence mutations detected in a cohort of more than 100 pediatric patients with major subtypes of AML (including FAB M7) at St. Jude Children’s Research Hospital. The affected genes are shown. ITD = internal tandem duplication.
Rational management of AML by monitoring in vivo early response to chemotherapy

In the absence of therapy directed to specific genetic subtypes of pediatric AML (with the exception of APL), clinicians have relied on patients’ early response to conventional myelosuppressive chemotherapy to guide subsequent treatment. Measurement of minimal residual disease (MRD) has been extraordinarily useful in the management of pediatric acute lymphoblastic leukemia (ALL), because multiparameter flow cytometry used in combination with molecular techniques allows reliable, reproducible measurement of residual disease in essentially all patients(23). However, it is more difficult to define an AML-specific marker profile to be monitored. In some cases, the flow cytometric features of the malignant cells are identical to those of their normal myeloid counterparts. Other cases are complicated by the existence of multiple minor clones. Therefore, flow cytometric residual disease monitoring is less sensitive in AML than in ALL; at St. Jude Children’s Research Hospital, residual AML is detected with a sensitivity of 0.1%, as opposed to 0.01% in ALL.

Quantitative and non-quantitative PCR-based methods have also been extensively used for MRD detection in AML(24). Quantitative reverse-transcription polymerase chain reaction (QRT-PCR) is highly sensitive, but its clinical utility is limited by the small number of subsets of pediatric AML that have leukemia-specific aberrations. Most of the clinically relevant data involve AML with the PML-RARα, CBFα-MYH11, and RUNX1-RUNX1T fusions, which indicate a favorable prognosis. In APL with the PML-RARα fusion, the goal of induction therapy is to eradicate MRD, as measured by PCR; the persistence or recurrence of MRD predicts a high risk of relapse. Importantly, most of the data in APL have been generated with non-quantitative PCR. In the case of pediatric AML with CBFα-MYH11 or RUNX1-RUNX1T, the clinical significance of residual fusion transcripts detected by non-quantitative PCR during complete clinical remission remains unclear. It is not uncommon for the RUNX1-RUNX1T marker to remain consistently detectable in children with AML who experience long-term clinical remission. It is not uncommon for the RUNX1-RUNX1T marker to remain consistently detectable in children with AML who experience long-term clinical remission. However, the persistence of any AML marker at a level =1% after two courses of induction chemotherapy, or the reappearance or consistent increase of MRD, as detected by any method at any time, is indicative of impending relapse. Continuing research is needed to address the limitations of current MRD assessment methods, and exciting

Table 1: Clonal Megakaryoblastic Disorders in Children

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Constitutional</th>
<th>Acquired</th>
<th>Disorder</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Down Syndrome (&lt;6 months of age)</td>
<td>Trisomy 21</td>
<td>GATA1 mutations</td>
<td>TMD</td>
<td>Excellent; spontaneous remission</td>
<td></td>
</tr>
<tr>
<td>Down Syndrome (6 months–4 years of age)</td>
<td>Trisomy 21</td>
<td>GATA1 mutations</td>
<td>AMKL</td>
<td>Favorable; cytarabine-based intensive chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Mosaic 21</td>
<td>GATA1 mutations</td>
<td>TMD/AMKL</td>
<td>Spontaneous remission; favorable</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Unknown</td>
<td>t(1;22); RBM15-MKL1</td>
<td>AMKL</td>
<td>Favorable; intensive chemotherapy</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Unknown</td>
<td>Trisomy 21 and other non-specific changes</td>
<td>AMKL</td>
<td>Poor prognosis</td>
<td></td>
</tr>
</tbody>
</table>

TMD: transient myeloproliferative disorder; AMKL: acute megakaryoblastic leukemia
new approaches are being explored(25, 26). Despite the methodological challenges, the available MRD methods can be integrated into the overall management plan for children with AML. In our opinion, the most useful aspect of such studies is their ability to indicate during early treatment whether a patient is unlikely to benefit from additional conventional chemotherapy.

Treatment of pediatric AML: lessons learned suggest a paradigm change

Until the late 1980s, all subtypes of pediatric AML were treated on the same protocols(27, 28, 29, 30, 31) The overall outcome of various treatment strategies was comparable, but the outcomes of specific AML subtypes differed among these studies, suggesting that some treatment components were more effective in selected subtypes. For example, some karyotypic abnormalities were associated with favorable prognosis in some studies but not others(10, 32, 33). During this period, two related types of AML, APL with the PML-RARα fusion and Down syndrome–associated AML, were shown to have unique clinical and biological features (34, 35, 36). Evidence that all-trans retinoic acid (ATRA) was effective in APL (in both children and adults) led to their exclusion from most front-line AML trials in favor of ATRA-based treatment strategies. Another factor was the observation that the most common cause of treatment failure in children with Down syndrome–associated AML was not resistant disease (as in other subtypes of AML) but treatment-related toxicity. Treatment strategies designed specifically for APL and Down syndrome–associated AML dramatically improved survival in both groups, but for different reasons. Survival in APL improved because of the availability of a highly effective drug that was systematically evaluated in different treatment phases, including induction, consolidation, and maintenance. Survival in Down syndrome–associated AML improved because of the feasibility of reducing treatment intensity, hence reducing the risk of toxic death, without compromising efficacy.

The aggregate analysis of studies in APL and Down syndrome AML strongly suggests that improvement of AML outcome may require clinical trials designed specifically for relatively homogeneous AML subtypes. Examples include infant AML, Down syndrome–associated AML, AML with the t(8;21), inv16, t(16;16), t(1;22), or t(9;11), and AML with a specific marker or mutation that can be therapeutically targeted. The APL success model, which reflects the collective effort of multi-institutional clinical and laboratory investigators who focused on this rare subtype of AML, could and should be applied to other subtypes. It may be unproductive to continue viewing AML as a single entity and enrolling all subtypes on a single protocol, as the efficacy of certain agents may not be detected, particularly those that are effective for rare subtypes of AML or for subsets of patients (e.g., those who have a specific genetic alteration) within more frequent subtypes(37). As a hypothetical example, a study designed to detect the benefit of ATRA in APL at the 5% probability level with 80% power in a classically-designed randomized study including all types of AML (92% non-APL, 8% APL) would have to accrue up to 6201 patients, which would take 19 years. However, if only APL patients were included, then the study would require enrollment of only 80 subjects and accrual could be completed in 2 years. These calculations assume that: i) the study would accrue 500 AML patients per year, ii) APL patients treated with standard therapy plus ATRA would have a 5-year EFS of 85%, iii) APL patients receiving standard therapy would have a 5-year EFS of 50%, and iv) non-APL patients receiving either therapy would have the same outcome as APL patients receiving standard therapy.

Components of treatment: a complex jigsaw puzzle with missing pieces

After treatment of thousands of children on AML trials over more than 4 decades, the essential components of AML therapy remain controversial. An analysis of contemporary pediatric AML trials in the US and Europe reveals enormous discrepancy in the number and type of drugs utilized, dosage and duration of treatment, strategy for CNS-directed therapy, intensity and duration of post-remission chemotherapy, indications for HSCT, and supportive care guidelines. Despite these differences, some general conclusions and opportunities for further investigation have emerged. First, induction chemotherapy must be intensive, leading to profound myelosuppression. Non-myeloablative remission induction is...
associated with poor outcome even when followed by intensive chemotherapy(38). The type, schedule, and dosage of remission induction agents have varied but have consistently included cytarabine and anthracyclines plus etoposide or 6-thioguanine. As long as profound myelosuppression is achieved, there is no evidence that any chemotherapy combination or schedule is superior to others, although most contemporary pediatric AML trials include cytarabine, daunomycin, and etoposide in the induction phase.

Intensive two-course induction chemotherapy in the context of optimal supportive care is associated with a 3% rate of treatment-related mortality and a 90% rate of complete morphologic remission in most centers in the developed world. Because the intensity of remission induction therapy is positively associated with improved overall survival, there have been several attempts to improve the therapeutic index of these regimens. Ideally, an added drug would be cytotoxic to the leukemia cells while sparing organs and normal hematopoietic tissue. Strategies that have been or are being investigated include increasing the dosage of cytarabine, using other anthracyclines (idarubicin or mitoxantrone) and nucleosides analogs (cladribine or clofarabine), and adding compounds that work by alternative mechanisms (gemtuzumab ozogamicin, FLT3 inhibitors). However, it is still too early to know whether these modifications improve the quality of remission induction and the overall survival rates.

There is consensus that additional chemotherapy is necessary after remission is induced. However, the type of post-remission therapy remains controversial(39, 40). In general, this phase of treatment is adapted to clinical and biological risk factors present at diagnosis, to whether there is persistent disease after induction therapy, and to the patient’s clinical performance status. In our just-completed AML02 protocol, patients with high-risk AML at diagnosis and evidence of persistent (=1%) leukemia after two courses of induction chemotherapy were candidates for allogeneic HSCT from related or alternative donors. Patients with favorable cytogenetic features and patients with intermediate-risk AML who did not have an HLA-matched sibling were treated with three courses of intensive post-remission chemotherapy. Those with intermediate-risk AML and an HLA-matched sibling underwent HSCT.

Indications for transplantation during first remission have been controversial(41-45). There is a broad consensus that HSCT reduces the rate of relapse but disagreement about whether it increases overall survival. This controversy is not surprising in view of the many factors that may influence the outcome of HSCT, including the health of the patient, biology of the AML, type of preparatory regimen, source of the stem cells, and post-transplant care. Patients’ pretransplant medical history, including number of courses of chemotherapy, severe complications requiring intensive care, latent fungal infection, and subclinical organ dysfunction, may influence transplant-related mortality. Moreover the experience of the center performing the transplant may affect its success(46, 47). Hence, optimal HSCT outcomes in AML may require the selection of patients for HSCT before they have been exposed to multiple courses of intensive chemotherapy, which can produce organ damage and selection of drug-resistant AML clones. We suggest that patients should be selected for eventual HSCT either at diagnosis (if they have high-risk features) or after remission induction therapy (if there is persistent AML). This strategy was used in the St. Jude AML02 protocol and resulted in a very low rate of transplant-related mortality; approximately two thirds of the transplant recipients are long-term survivors (unpublished data). Another center has reported similar results in patients with intermediate-risk AML in first complete remission who were selected to undergo HSCT if an HLA-matched sibling donor was available(48).

For patients selected to receive post-remission chemotherapy, the controversy has centered on the number of courses needed and whether prolonged non-myelosuppressive chemotherapy is needed. The number of post-remission courses has been evaluated by Medical Research Council investigators(40). In patients younger than 55 years of age, five courses in total were not more advantageous than four courses, even when the HSCT was included. In a retrospective subgroup analysis, patients who had three courses fared as well overall as those who received four
courses. However, in patients with high-risk AML, four courses were superior to three courses. The duration and type of intensive post-remission chemotherapy has not been formally studied in pediatric AML. Most contemporary pediatric AML trials feature two to four courses of intensive post-remission therapy. There is no evidence that prolonged non-myeloablative chemotherapy provides a benefit except in APL, in which the combination of daily 6-mercaptopurine (6-MP) with weekly methotrexate (MTX) plus intermittent ATRA was superior to both 6-MP plus MTX and observation alone.

CNS-directed therapy has not been prospectively studied in pediatric AML. Approximately 17% of patients have CNS involvement at diagnosis, and the rate of CNS relapse (isolated or non-isolated) ranges from 3% to 8%. Risk factors for CNS relapse include young age, leukocytosis, monoblastic subtype, and MLL rearrangement. CNS-directed therapy has consisted of cytarabine alone, cytarabine plus MTX, or MTX plus hydrocortisone. Indications for CNS radiotherapy in AML remain controversial, but there is increasing evidence that it offers little or no benefit in controlling CNS involvement. In our two recent St. Jude studies, AML97 and AML02, radiotherapy was not prescribed for newly diagnosed patients. Four monthly triple intrathecal (TIT) injections of MTX, cytarabine, and hydrocortisone were given to children without CNS leukemia at diagnosis. Those with CNS involvement at diagnosis received four weekly TIT injections followed by four monthly TIT injections. The CNS relapse rate was less than 3% in these two studies.

Supportive care in AML: a neglected area of research

Treatment-related mortality and morbidity is a major challenge in pediatric AML. Progress in supportive care is considered one of the most important contributors to improved survival in contemporary AML trials. A multidisciplinary approach that includes easy access to intensive care units, newer resuscitation techniques, and liberal use of blood products, prophylactic antibiotics, and antifungals has dramatically improved the outcome of patients with AML. However, there have been few well-designed studies to elucidate the mechanism of early complications and find ways to improve supportive care in AML. Moreover, the number of early deaths in AML is difficult to quantify because many patients are not eligible for enrollment on protocols. Among those enrolled, early mortality has ranged from 2% to 7%. Risk factors for early mortality include leukocytosis, age <2 years, monoblastic leukemia, and APL.

It is likely that individualized approaches based on the leukemia subtype will be necessary to reduce early mortality. As proof of concept, early mortality in patients with APL has been reduced by using an individualized approach to reduce bleeding complications and differentiation syndrome. Patients with monoblastic leukemia typically develop severe systemic inflammatory response and multiple organ damage when exposed to antimetabolites, including cytarabine, regardless of their initial leukocyte count. AML with the inv16 appears to predispose patients to pulmonary complications. In these cases, measures to protect the respiratory, cardiovascular, and renal systems while the patient receives adapted doses of chemotherapy (preferably continuous-infusion cytarabine at 100 mg/m²) allow gentle reduction of the leukemia burden while preserving organ integrity. Because of differences in cellular metabolic activity and the difficulty of measuring tumor burden, the indications for leukapheresis continue to be controversial in AML, although leukapheresis is commonly performed for patients with a white blood cell count >100,000 x 10⁹/L. Hydroxyurea has also been shown to reduce hyperleukocytosis in AML, but its role remains unresolved.

After remission is induced, most deaths that are not caused by AML are caused by infection. Broad-spectrum antibacterial and antifungal agents have substantially decreased the frequency of fatal infections in these patients.

Summary

With current treatment strategies, only about 60% of the children diagnosed with AML between 2005 and 2009 are expected to be alive in 10 years. We believe that survival rates can be increased to equal those obtained in APL, which until recently was considered the deadliest form of AML. The current one-size-fits-all paradigm for the
management of pediatric AML must be challenged. Instead, we suggest a model in which clinical and laboratory investigators collaborate to produce breakthroughs such as that achieved in APL. Because of the small number of pediatric AML cases and the multiple biologic AML subgroups, meticulously organized national and international collaborative trials should be conducted with the ultimate aim of proving that pediatric AML need not be an ominous diagnosis.

References


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Prognostic Factors in Wilms Tumor of the Kidney

Paul Grundy

Abstract

The outcome is now good for most patients with Wilms tumor of the kidney. Over and above the gains in survival, the ability to progressively regulate the amount of chemotherapy and radiation so that groups of patients are receiving dosing adequate to achieve cure, but not more, has been made possible by the use of prognostic factors. We often now think of prognostic factors as molecular or biologic findings, but factors used to predict outcome in patients with Wilms tumor—to thereby stratify therapy—include histology (favourable versus anaplastic), stage (using criteria such as lymph node involvement, local or intravascular tumor extension and presence of metastatic disease), age at diagnosis, response to therapy, and now molecular or genetic changes (Loss of Heterozygosity (LOH) for chromosomes 1p and 16q).

Prognostic factors are determined retrospectively and must always be validated in a second population of patients. Furthermore, prognostic factors are dependent on the treatment used in the population in which they are identified. Thus as therapy changes from study to study, so too can prognostic factors so they must be constantly re-assessed.

Examples of these principles are discussed and information on current prognostic factors being utilized is presented.

Introduction

The National Wilms Tumor Study Group (NWTSG) has completed five clinical trials (1-6) during which time the survival rate of children with Wilms tumor has increased from 20%, prior to the availability of effective chemotherapy, to almost 90% (5). Notably, the improvement in outcome has been achieved while actually using shorter duration and total amount of chemotherapy and lower doses of radiation (4,5). Current trials are examining the benefit of intensified chemotherapy for specific subsets of patients, while maintaining overall reduced doses for most, and attempting to eliminate adjuvant therapy for one small subset of patients.

Over and above the gains in survival due to the advent of effective chemotherapy, the ability to progressively regulate the amount of chemotherapy and radiation so that groups of patients are receiving dosing adequate to achieve cure, but not more, has been made possible by the use of prognostic factors. We often now think of prognostic factors as molecular or biologic findings, but factors used to predict outcome in patients with Wilms tumor—to thereby stratify therapy—include histology (favourable versus anaplastic), stage (using criteria such as lymph node involvement, local or intravascular tumor extension and presence of metastatic disease), age at diagnosis, response to therapy, and now molecular or genetic changes, (Loss of Heterozygosity (LOH) for chromosomes 1p and 16q). The purpose of this paper is to review these prognostic factors, the practical changes which have resulted from their use, and to speculate on possible future prognostic factors. As we have extensively studied clinical parameters, additional prognostic factors will most likely be biological in nature.

Stage

The first prognostic factor used in NWTS-1 was tumor stage (stage was called group in NWTS-1) which was assigned according to the extent of disease prior to the administration of chemotherapy (Table 1)(1). In fact, the very first staging system was developed somewhat empirically (although logically) since there was no prior large series of cases to analyse to establish or validate the importance of the various
staging criteria. The outcomes in NWTS-1 however, did validate the staging criteria in general. Patients in Group I had a significantly better outcome compared with similarly treated Group II/III patients. There was no apparent difference in outcomes between Group II and III though.

**Table 1: Clinical Grouping**

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Tumor limited to kidney and completely resected. The surface of the renal capsule is intact. The tumor was not ruptured before or during removal. There is no residual tumor apparent beyond the margins of resection.</td>
</tr>
<tr>
<td>Group II</td>
<td>Tumor extends beyond the kidney but is completely resected. There is local extension of the tumor; i.e., penetration beyond the pseudocapsule into the peri-renal soft tissues, or peri-aortic lymph node involvement. The renal vessels outside the kidney substance are infiltrated or contain tumor thrombus. There is no residual tumor apparent beyond the margins of resection.</td>
</tr>
<tr>
<td>Group III</td>
<td>Residual nonhematogenous tumor confined to abdomen. Any one or more of the following occur: 1) The tumor has been biopsied or ruptured before or during surgery; 2) there are implants on peritoneal surfaces; 3) there are involved lymph nodes beyond the abdominal peri-aortic chains; 4) the tumor is not completely resectable because of local infiltration into vital structures.</td>
</tr>
<tr>
<td>Group IV</td>
<td>Hematogenous metastases. Deposits beyond Group III; e.g., lung, liver, bone and brain.</td>
</tr>
<tr>
<td>Group V</td>
<td>Bilateral renal involvement either initially or subsequently.</td>
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</table>

This study, which utilized a single chemotherapeutic agent, Actinomycin D, for Group I patients and for a randomized subset of Group II/III patients, demonstrates two principles of the use of prognostic factors. There may be interactions between factors, and these interactions are dependent on the treatment utilized. In Group I patients, treated only with Actinomycin D, there was a clear effect of age with those less than age 2 years having a significantly better outcome than those greater than age 2 (1). Interestingly, this same phenomenon has been reported by the United Kingdom Children’s Cancer Study Group (UKCCSG) who observed better outcomes for children with stage I tumors treated with Vincristine only if under age two at diagnosis (7). This effect of age was not observed in Group II/III children, nor has it been found to be an independent prognostic factor in subsequent NWTSG studies (unpublished data) all of which have used combination chemotherapy. I believe this demonstrates the principle that the prognostic power of any given factor is dependent on the treatment context. In this example, age at diagnosis is predictive of outcome, but only in the context of minimal therapy. With more effective therapy, this predictive effect is lost.

We also see in NWTS-1 that patients with Group II/III tumors treated with the combination of Actinomycin D and Vincristine actually fared better than the subset of patients who were older than age two with Group I disease treated with Actinomycin D only and without radiation. These data do not invalidate the staging/grouping system but demonstrate the interactions of multiple prognostic factors and the therapeutic context. These conclusions may seem self evident, but particularly when complex, state of the art molecular genetic assays are proposed as predictors, these simple tenets are often forgotten.

Since prognostic factors are identified retrospectively, their significance may change when more effective treatment regimens are developed. This in fact has occurred with the sequential evaluation of prognostic factors among children treated on the National Wilms Tumor
Studies. The current definitions are listed in (Table 2). The most significant recent change has been in the distinction between Stages I and II(8). Prior to NWTS-5, one of the criteria for Stage II included extension of the tumor past the hilar plane, an imaginary boundary marked by the medial border of the renal sinus. The renal sinus is biologically quite important because it contains the major renal vessels, a potential route for hematogenous and lymphatic spread. However, this “hilar plane” has proven difficult to reliably and objectively assess and so the hilar plane criterion for staging was removed from NWTS-5, and replaced by the criterion of renal sinus vascular invasion which is assessed microscopically. Applying the new criteria, the difference in survival between Stage I and II Wilms tumors continues to be statistically significant.

Table 2: Current staging criteria used by the Children’s Oncology Group

<table>
<thead>
<tr>
<th>Staging</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>Tumor limited to kidney, completely resected. The renal capsule is intact. The tumor was not ruptured or biopsied prior to removal. The vessels of the renal sinus are not involved. There is no evidence of tumor at or beyond the margins of resection. NOTE: For a tumor to qualify for certain therapeutic protocols as Stage I, regional lymph nodes must be examined microscopically.</td>
</tr>
<tr>
<td>Stage II</td>
<td>The tumor is completely resected and there is no evidence of tumor at or beyond the margins of resection. The tumor extends beyond kidney, as is evidenced by any one of the following criteria: • There is regional extension of the tumor (i.e. penetration of the renal capsule, or extensive invasion of the soft tissue of the renal sinus, as discussed below) • Blood vessels within the nephrectomy specimen outside the renal parenchyma, including those of the renal sinus, contain tumor. Note: Rupture of spillage confined to the flank, including biopsy of the tumor, is no longer included in Stage II and is now included in Stage III.</td>
</tr>
<tr>
<td>Stage III</td>
<td>Residual nonhematogenous tumor present following surgery, and confined to abdomen. Any one of the following may occur: • Lymph nodes within the abdomen or pelvis are involved by tumor. (Lymph node involvement in the thorax, or other extra-abdominal sites is a criterion for Stage IV) • The tumor has penetrated through the peritoneal surface, • Tumor implants are found on the peritoneal surface, • Gross or microscopic tumor remains postoperatively (e.g., tumor cells are found at the margin of surgical resection on microscopic examination), • The tumor is not completely resectable because of local infiltration into vital structures, • Tumor spillage occurring either before or during surgery, • The tumor is treated with preoperative chemotherapy (with or without a biopsy regardless of type- tru-cut, open or fine needle aspiration) before removal, • Tumor is removed in greater than one piece (e.g. tumor cells are found in a separately excised adrenal gland; a tumor thrombus within the renal vein is removed separately from the nephrectomy specimen). Extension of the primary tumor within vena cava into thoracic vena cava and heart is considered Stage III, rather than Stage IV even though outside the abdomen.</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Hematogenous metastases (lung, liver, bone, brain, etc.), or lymph node metastases outside the abdominopelvic region are present. (The presence of tumor within the adrenal gland is not interpreted as metastasis and staging depends on all other staging parameters present).</td>
</tr>
<tr>
<td>Stage V</td>
<td>Bilateral renal involvement by tumor is present at diagnosis. An attempt should be made to stage each side according to the above criteria on the basis of the extent of disease.</td>
</tr>
</tbody>
</table>
Another major change in the staging criteria for the current Children’s Oncology Group (COG) studies is the categorization of tumor spill. In prior NWTS studies, tumor spill was classified as “local” – a criterion for stage II, or “diffuse” – a criterion for stage III. The difficulty was that although one could provide examples which everyone would agree represented either a local or diffuse spill, there was no objective definition of the difference between the two. Furthermore, a study of NWTS-4 patients led by John Kalapurakal showed that patients with stage II disease with local spill had lower 8-year relapse-free survival – 79% vs 87% (p=0.07) and overall survival – 90% vs 95% (p=0.04) compared to stage II patients without spill when treated without abdominal radiation therapy(9). It was thus concluded that the group of patients with any form of intraoperative spill would have a better outcome if treated with irradiation (as stage III) and spill was then changed to a criterion for stage III. It will be difficult to prove that this change is beneficial. Certainly we can monitor the outcomes of patients on the current trials who are stage III for spill alone – but not only is this small group, and a single arm study, but the distribution of children whose spill might have been classified as diffuse vs local will be impossible to know.

**Histology**

The histologic finding of anaplasia was first identified as an important determinant of prognosis by Beckwith and Palmer in 1978(10). The criteria for the diagnosis of anaplasia include: 1) the identification of nuclei with a diameter at least three times those of adjacent cells, 2) hyperchromasia of the enlarged cells providing evidence for increased chromatin content; and 3) the presence of multipolar or otherwise recognizably polyploid mitotic figures(11). Anaplasia is not common, approximately 5% of all Wilms tumors, and correlates with patient age. It is rare in the first 2 years of life and then increases to a relatively stable rate of about 13% in patients older than 5 years.

Anaplasia has remained the most adverse prognostic factor since being defined. The results in Table 3 show the value of identifying this small subset(12). While overall treatment intensity has been reduced for patients with favourable histology Wilms tumor, these data have allowed the sequential intensification of therapy – adding doxorubicin, cyclophosphamide and now etoposide – with modest but definite improvements in outcome. Without this factor these children would still be included in the larger group and would not have benefited from improvements in therapy.

<table>
<thead>
<tr>
<th>Stage</th>
<th>4 yr relapse-free survival % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reg DD4A RT VDA</td>
</tr>
<tr>
<td>II</td>
<td>40 (12)</td>
</tr>
<tr>
<td>III</td>
<td>33 (9)</td>
</tr>
<tr>
<td>IV</td>
<td>0 (8)</td>
</tr>
</tbody>
</table>

Other more limited correlations between outcome and histology have been reported. Blastemal rich tumors tend to be extremely invasive but often respond well to chemotherapy. In contrast, predominantly epithelial and rhabdomyomatous Wilms tumors more frequently present at a low stage, reflecting less aggressiveness, yet are often resistant to chemotherapy. While these prognostic trends have been observed and attempts made to exploit them, it has not been possible to define them in such a way as to show reproducible significant differences in outcome and so they have not been incorporated into staging or risk–based schemas.

**Age and Tumor weight**

Several reports have shown that the outcomes for children < 2 years of age at diagnosis with Stage I favorable histology Wilms tumors that weigh < 550 g have been excellent and not apparently influenced by treatment(13). In NWTS-5 these patients did not receive any post-
operative chemotherapy or radiotherapy. An early stopping rule was triggered when the 3-year interim analysis showed a 2-year event free survival of 86.5%. Unfortunately we had conservatively designed the trial with the assumption that only 50% of the patients with recurrence would be successfully salvaged, whereas in reality, 10 of the eleven children whose tumor recurred were salvaged. Further review of the relapsed cases suggested that central pathological review (to confirm pathological stage I) and inclusion of stage I tumors only with known lymph node status might further reduce the rate of recurrence after surgery only. This study is therefore being carried out currently in the COG with this subset designated “Very Low Risk”.

Response of the tumor

SIOP investigators have long based the need for whole lung irradiation for pulmonary metastases on whether there is a complete response to the first six weeks of chemotherapy. Seventy five percent of patients with Stage IV FH WT treated on SIOP trials have thus been spared whole-lung irradiation with apparently comparable outcomes to the NWTSG(14). These results cannot be immediately utilized in North America because the chemotherapy regimen used by SIOP is more intensive, particularly with regards to anthracycline dosage. It is therefore possible that with holding radiation in the context of the lesser chemotherapy regimen used by COG might result in more recurrences. This question is being currently examined in the COG trial, with the further differences that complete response is being defined by CT scan at week six, rather than by chest X-ray, and that the response must be attained following chemotherapy only, not surgery. These criteria are therefore more conservative than the SIOP approach. Finally, COG is using the failure to attain a complete response as a predictor of adverse outcome and is testing the intensification of chemotherapy on this subset of patients.

The use of pre-operative chemotherapy has been studied and refined by SIOP investigators. This provides the opportunity to assess tumor response – whether by volume reduction (imaging) and by histological response. Tumor volume reduction, although clearly associated with outcome, must be correlated with predominant histology. For example, rhabdomyomatous or stromal predominant tumors may not shrink at all in response to chemotherapy yet have a favourable outcome if resected completely. Although this correlation exists, criteria have not yet been defined which allow the incorporation of volume reduction into treatment-determining risk strata.

The histological response of the tumor after pre-operative chemotherapy has also been shown to be prognostic. Patients with completely necrotic tumors after four weeks of therapy have an excellent outcome, and when stage I require very little if any postoperative chemotherapy(15;16). Conversely, blastemal predominant tumors post chemotherapy appear to be high risk – comparable with anaplastic tumors and intensified therapy is being investigated for this subset in SIOP 2001.

Biology and Genetics

In 1994, Pediatric Oncology Group investigators showed that, among 232 children with Wilms tumor registered on NWTS - 3 and - 4, loss of heterozygosity (LOH) for polymorphic DNA markers on chromosome 16q, present in tumor tissue from 17.2% of those with favorable or anaplastic histology tumors, was associated with statistically significantly poorer two-year relapse-free and overall survival percentages even when adjusted for stage or histology (17). LOH for chromosome 1p, present in tumor tissue from 11% of children was also associated with poorer relapse-free and overall survival although these results were not statistically significant. By contrast, LOH for 11p, a region thought to contain at least two Wilms tumor-related genes, found in 33% of cases, was not associated with any difference in outcome (17).

NWTS-5 was designed to prospectively test this proposed association between LOH for chromosome 16q or chromosome 1p and outcome in favourable histology Wilms tumor, all of whom were treated with common, stage-specific treatment regimens. The study was designed to detect clinically significant associations within stages of disease; namely stages I/II and III/IV and to more provide more accurate estimates of the outcomes. As shown in Tables 4 and 5, patients with Stage I/II tumors
had worse outcomes with either LOH chromosome 1p or 16q but it was the subset with LOH of both which had a three fold risk of relapse, which equated to a 75% relapse-free survival, a clinically significant difference relative to those with LOH for neither chromosome who had a 90% RFS. Similarly, for stage III/IV patients, those with LOH for 1p and 16q had a 2.5 increased risk of relapse and death – a 65% RFS compared with 83% for those without LOH. This has now been added to stage and histology to generate “Risk” groups (Table 6). It is important to note that although LOH is now proven to be associated with an adverse outcome, that intensified therapy has not yet been shown to improve relapse-free or overall survival. That is one of the questions of the current COG trials.

Table 4: RFS and OS by joint LOH at chromosomes 1p and 16q for clinical stage I/II favorable histology Wilms tumor patients

<table>
<thead>
<tr>
<th>LOH status</th>
<th># pts</th>
<th># relapses</th>
<th>4 yr RFS%</th>
<th>RR(95% C.I.)</th>
<th># deaths</th>
<th>4 yr OS%</th>
<th>RR(95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither</td>
<td>750</td>
<td>60</td>
<td>91.2</td>
<td>——-</td>
<td>14</td>
<td>98.4</td>
<td>——-</td>
</tr>
<tr>
<td>1p only</td>
<td>60</td>
<td>11</td>
<td>80.4</td>
<td>2.19(1.15-4.17)</td>
<td>p=0.02</td>
<td>4</td>
<td>91.2</td>
</tr>
<tr>
<td>16q only</td>
<td>114</td>
<td>19</td>
<td>82.5</td>
<td>1.91(1.14-3.21)</td>
<td>p=0.01</td>
<td>3</td>
<td>98.1</td>
</tr>
<tr>
<td>Both</td>
<td>46</td>
<td>11</td>
<td>74.9</td>
<td>2.88(1.51-5.49)</td>
<td>p=0.001</td>
<td>4</td>
<td>90.5</td>
</tr>
</tbody>
</table>

*RR’s are calculated with stratification on stage I/Age<24m/Wt<550g, stage I/Age>=24m or Wt>=550g, and stage II

Table 5: RFS and OS by joint LOH at chromosomes 1p and 16q for stage III/IV favorable histology Wilms tumor patients

<table>
<thead>
<tr>
<th>LOH status</th>
<th># pts</th>
<th># relapses</th>
<th>4 yr RFS%</th>
<th>RR(95% C.I.)</th>
<th># deaths</th>
<th>4 yr OS%</th>
<th>RR(95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neither</td>
<td>500</td>
<td>82</td>
<td>83.0</td>
<td>——-</td>
<td>38</td>
<td>91.9</td>
<td>——-</td>
</tr>
<tr>
<td>1p only</td>
<td>56</td>
<td>6</td>
<td>89.0</td>
<td>0.69(0.30-1.57)</td>
<td>p=0.37</td>
<td>2</td>
<td>97.6</td>
</tr>
<tr>
<td>16q only</td>
<td>100</td>
<td>15</td>
<td>85.3</td>
<td>0.89(0.51-1.54)</td>
<td>p=0.67</td>
<td>7</td>
<td>92.0</td>
</tr>
<tr>
<td>Both</td>
<td>30</td>
<td>9</td>
<td>65.9</td>
<td>2.41(1.20-4.82)</td>
<td>p=0.01</td>
<td>5</td>
<td>77.5</td>
</tr>
</tbody>
</table>

*RR’s are calculated with stratification on stage III and IV
Messahel has reported on the prognostic significance of loss of heterozygosity (LOH) on 1p and 16q in 426 favourable histology Wilms tumours treated with either immediate nephrectomy (63%) or preoperative chemotherapy (37%) in the UK. Intriguingly, although they found the same incidence of LOH 1p and 16q as in the NWTS series, only LOH 16q was associated with an increased risk of relapse (hazard ratio (HR) 2.69, 95%CI: 1.47-4.92) and death (HR 2.67, 95%CI: 1.17-6.06) while LOH 1p showed no significant associations. Whether this difference between the North American and UK results reflects the inability of the smaller UK series to detect a significant association with 1p LOH (the first NWTS study which was also smaller also showed 16q but not 1p to be associated with adverse outcome) or a difference in the biology of Wilms tumors in different populations is not known.

Another prognostic marker for favorable histology Wilms tumor is the expression level of telomerase in the tumor. Telomerase, which plays a key role in cellular immortalization, includes a catalytic subunit (TERT), and an RNA subunit (TERC/hTR). A small case-cohort study involving 78 NWTS-5 registrants revealed a positive correlation between tumor expression level of TERT mRNA and the risk of tumor recurrence. (18) A follow-up study involving 296 patients confirmed the correlation between TERT expression and recurrence, but found that TERC expression was actually the more powerful predictive assay(19). Although statistically significant, and independent of stage and LOH status, the outcome for patients in the adverse group was still 80%. Future studies will therefore be needed to evaluate how telomerase expression may be used in conjunction with other prognostic markers, such as LOH at 1p and 16q, since the group identified by high telomerase expression does not have an adverse enough outcome to warrant alteration of treatment for that factor.

Other published reports provide potential evidence for the prognostic import of alteration in copy number of several other chromosomal loci in Wilms tumors. The strongest evidence supports the importance of increased copy number of chromosomes 1q and of 15q (at the insulin-like growth factor receptor (IGF1R) locus)(22), and deletion of 11q (23) while there is weaker but suggestive evidence for several other chromosomal locations (7p, 12p, 22q). A
large comprehensive study capable of examining alterations at all these loci is needed to validate each and to determine interactions between them.

The feasibility and potential clinical utility of classifiers of relapse based on global gene expression analysis have been reported by several authors but the largest was undertaken by Perlman using NWTS samples(24). Gene expression was profiled for two hundred fifty favourable histology Wilms tumors of all stages enriched for relapses treated on National Wilms Tumor Study-5. For stage I/II tumors, the number of genes associated with relapse was less than that predicted by chance alone. This suggested very little likelihood of identifying true predictors and no further analyses were done. However for stage III patients the initial study and cross-validation including an additional 68 patients showed that classifiers for relapse composed of 50 genes were associated with a median sensitivity of 47% and specificity of 70%. This study shows the feasibility and modest accuracy of stratifying local stage III FHWT using a classifier of <50 genes although further validation is still needed as would be the validation of a more practical assay. Of course, these results also underline the issue of heterogeneity in the cause of relapse.

Conclusions

It is likely that we have almost exhausted the generation of clinical indicators of outcome, except perhaps for refinements of tumor response – by imaging or histology. More likely, will be the development of assays of gene expression, perhaps indicating the activation of cell cycle regulatory pathways, or the inactivation of apoptosis. Current chip technology is very useful for first identifying patterns of gene expression but ultimately these need to be reduced to more simple reproducible (and less expensive) assays which can be carried out routinely in clinical laboratories. It also seems clear that no one assay will be sufficient. I expect that we will see modifications of current “Risk” groups with more and more – hopefully more specific – criteria used to assign Risk.

References


Surgery For Rare Tumors In Children: The experience of the Italian TREP Project

Giovanni Cecchetto

Abstract
A common definition of rare tumors in children is not accepted everywhere. The Italian TREP Project, which started in 2000, defined as rare tumors those malignancies characterized by an annual incidence of less than 2 per million children and adolescents up to age 18, and not included in other clinical trials. This group includes a heterogeneous variety of solid tumors with different characteristics, which requires appropriate clinical approach. Usually, many of these tumors are initially observed in pediatric surgery centers, and every pediatric surgeon may encounter a rare tumor during his activity.

The awareness of the existence of rare tumors is important for a correct diagnostic work-up and treatment. Pediatric surgeons must know role and timing of surgical therapy: several solid rare tumors may require surgical treatment alone, (adrenocortical tumors, renal carcinoma, gonadal non germ cell tumors, pheochromocytoma); others, such as pleuropulmonary blastoma, nasopharyngeal carcinoma need a multidisciplinary therapy. Therefore pediatric surgeons have to collaborate with other specialists. The various histotypes, furthermore, arise in different body sites (cutaneous tumors, abdominal tumors, thyroid carcinoma...): surgeons with appropriate expertise are needed. Finally, some tumors are exceptional in childhood, whereas they are very common in adults (colon cancer, breast cancer): in these cases the task for a pediatric surgeon is to recognize the disease, while the surgical treatment should be performed together with surgeons for adults. All these issues have been faced in the TREP Study.

An international cooperative Group including oncologists, surgeons, and other specialists is warranted to improve studies on rare tumors.

Introduction
The concept of rare solid tumors is not clearly defined in children. A tumor may be very rare in childhood, less infrequent in adolescents but very common in the adult population, or it may be rare in all age groups. Since epidemiologic, clinical and histological data are generally few and sparse, clinicians (even those working in important Centers) are often unfamiliar with the management of these diseases, and usually a common clinical approach does not exist. For this reason, rare tumors are considered “orphan” diseases: as a consequence, shared treatment guidelines are difficult to promote.

The involvement of pediatric surgeons is very important, because in many cases rare tumors are initially observed in Pediatric Surgery Centers, and sometimes surgery is the only treatment. The role of the pediatric surgeons, especially those working in major institutions, is twofold: on one hand they should know the issue and what to do, because some tumors are treated with chemotherapy (CT), some with surgery only, and some need a multidisciplinary therapy; on the other hand they should know the specific surgical approach in different situations.

Purpose of this article is not to provide exhaustive information on all rare tumors, but rather to focus the role of pediatric surgeons for some rare tumors for which surgery represents the main treatment. Concepts and data have been obtained from the Italian Study on Rare Tumors (TREP Project).

Pediatric Surgeons & The Italian TREP Project
The Italian Cooperative Project called “Tumori Rari in Età Pediatrica” (TREP) was launched in 2000 under the auspices of the Associazione Italiana di Ematologia Oncologia (AIEOP), and the Società Italiana di Chirurgia Pediatrica (SICP). Italian Pediatric Surgeons were involved from the beginning and some of them were the promoters.
of the project. Actually they had previously collaborated in a retrospective study on 259 patients up to age 16 affected by rare tumors observed in Italian Pediatric Oncology and Surgery centers between 1982 and 1998.

The TREP Group defined as “rare” any solid tumor characterized by an annual incidence of <2 per million children and adolescents up to age 18, and not included in other clinical trials (malignant germ cell tumors and hepatic tumors are rare but have their own protocols, some rare non rhabdo-soft tissue sarcomas are registered in cooperative studies...). This definition mainly considered practical and clinical issues rather than epidemiologic data and included a heterogeneous group of tumors, with different sites and clinical behaviour which require different experts (oncologists, surgeons, pathologists, biologists).

The following entities were included in the study: nasopharyngeal carcinoma, adrenocortical tumours, pleuro-pulmonary blastoma (and other lung tumours), carcinoid tumours, skin cancer (cutaneous melanoma and al.) renal cell carcinoma, pancreatoblastoma (and other pancreatic exocrine tumours), gonadal non-germ-cell tumours (ovary/testis), pheochromocytoma and paraganglioma, thyroid carcinoma, other rare tumors (salivary gland tumours, breast carcinoma, gastrointestinal carcinoma,...).

The main aims of the project were 1) to register and collect clinical data; 2) to develop, on the basis of the international literature, diagnostic and therapeutic recommendations for every histotype, that could assist physicians and patients in deciding the appropriate approach to specific clinical conditions; 3) to define a collaborative network with other specialists (i.e. adult oncologists and surgeons); 4) to identify one or two “expert” colleagues to be in charge for each histotype and to be contacted by other clinician in case of problems with a little patient affected by that specific rare tumors; 5) to promote pathological and biological studies.

A strict collaboration with a Panel of Pathologists on the revision of the diagnosis and possible studies was considered necessary from the beginning of the study. A central review allowed a certain diagnosis, the study of histopathological characteristics and the comparison with similar tumours in adults. Specific biological studies started (for instance when a pheochromocytoma was diagnosed, centres were asked to send their patient’s blood samples to a reference laboratory to check for VHL syndrome). The Group was able to involve some Specialists in other disciplines, such as endocrinologists, radiotherapists, plastic surgeons, general surgeons for adults, who often observe cases of adolescents with specific adult tumors (thyroid carcinoma, melanoma, bowel carcinoma and salivary gland tumors).

The registration of cases up to now has been satisfactory. Fig.1 summarizes the tumor types registered in the TREP Study from January 2000 to March 2009.

![Fig.1: Tumor types registered in the Italian TREP Project](image-url)
The guidelines for 12 histotypes were prepared with the collaboration of physicians from various Italian centers (mainly pediatric oncologists and pediatric surgeons), who periodically revise them on the base of new literature. Those physicians are also available for consult.

The challenge has been to maintain update the guidelines and verify their value analysing the outcome of the patients treated according to them. In the last years, most patients have been treated according to the guidelines. An analysis conducted in 2007 by Pastore et al. to compare the number of patients enrolled with the number of rare tumors expected to be diagnosed, demonstrated that for the patients aged 0-14 the ratio of observed to expected cases was 1:1, showing that the vast majority of patients are registered and treated according to TREP protocol recommendations, and confirming the feasibility of multicenter studies. Moreover the incidence of several histotypes, among the ages 15 to 17, was more than 2/million.

Pediatric surgeons collaborated in the recruitment of patients and were strictly involved in preparing diagnostic/therapeutic recommendations. We believe that the achievement of a good cooperation with other specialists has been an important objective. Fig. 2 show that about 30% of patients were diagnosed in pediatric surgery centers. Some rare tumors that require different surgical skills and for which surgery plays an important role will be discussed in the next paragraph.

![Fig. 2: TREP Project: registration by center](image)

**Surgery For Specific Rare Tumors**

**Adrenocortical Tumors.** Adrenocortical tumors (ACT) represent about 0.2% of all pediatric malignancies and 5-6% of all adrenal tumors. The incidence varies across geographic regions and is remarkably high in southern Brazil. The age incidence curve is characterized by two peaks, the first being under 3 years of age and the second during adolescence. Some associated syndromes are described, such as the Beckwith-Wiedemann syndrome, Gardner syndrome and the familiar Li-Fraumeni syndrome, that includes various forms of familial tumors. In these syndromes it has been found that ACT are associated to p53 mutation; most children (80%) have secreting tumors, and signs of virilization are the most frequent findings. About 10% of the patients do not present endocrine symptoms, and the lesion may manifest as an abdominal mass or it may be found occasionally. Tumor size, in term of weight or volume or diameter, is traditionally considered a prognostic factor, and in literature various values have been used as discriminator, being a tumor weight of less than 200 grams or a volume of less than 200 cm³ linked to a favorable prognosis. Regarding the treatment, surgery represents the main step. The need for complete surgical excision has been described by many studies. Perioperative steroid administration may be required due to a suppressed hypotalamic-pituitary-adrenal axis. An open procedure is recommended. A miniinvasive surgical approach, generally adopted in adults, is accepted only for small tumors in selected centers. The goal is the achievement of a microscopical complete surgery, which avoids the risk of local relapse and correlates with favourable prognosis. The para-aortic and para-caval regions have to be explored and any doubtful lesion and enlarged lymph-nodes to be excised. The contemporary removal of the kidney if involved, is accepted also in primary surgery.
Delayed resection of the primary tumor and/or metastases are suggested in initially inoperable cases. CT and Mitotane may be utilized in non completely excised cases and for metastatic disease, even if results are controversial. The distinction between adenoma and carcinoma has been a matter of concern, because their common clinical and histological characteristics. Malignant lesions are generally larger in size and more invasive. Many studies have investigated histological criteria of malignancy and some immunohistochemical markers of aggressive behaviour in adults, allowing predicting outcome: in particular, metalloproteinasis 2 (MMP2) has shown a strong relationship with an unfavorable prognosis in this age group. However, in children, due to the rarity of these lesions, it has been not possible to define true characteristics of malignancy so far.

Between January 2000 and March 2009, 32 adrenocortical tumors have been registered in the TREP Study: 20 patients in St.I (complete excision of tumors <200cm³) are in complete remission, 2 of 7 in St.II (complete excision of tumor >200cm³ or micro residuals or tumor spillage) died of disease, 1 in St.III (initial biopsy or excision with macro residuals) is in therapy, alive with disease, 4 in St.IV (metastatic disease) died of disease.

**Pheochromocytoma.** It is a tumor arising from adrenal medulla. When it develops from extraadrenal locations (30% of cases) it is known as paraganglioma. Pheochromocytomas commonly synthesize catecholamines which cause continuous or paroxysmal arterial hypertension, refractory to conventional treatment. Pheochromocytoma may be sporadic but develops also in association with MEN II syndrome (multiple endocrine neoplasia), Von Hippel-Lindau disease and neurofibromatosis type I. When the diagnosis has been obtained, treatment with adrenergic antagonists is required for preoperative preparation, to reduce blood pressure. Complete removal of the tumor is the treatment of choice and the outcome after surgery is excellent since only 10% of pheochromocitomas are malignant. In patients with unresectable or metastatic forms, the disease can be managed with medical treatment but the results are unsatisfactory.

Up to now 19 cases have been enrolled in the TREP Study: 13 pheochromocitomas, 6 paragangliomas. Fourteen cases underwent primary complete excision and all of them are alive and well. Also 2 patients with suspected microscopical residuals are in complete remission without other treatment. Chemotherapy obtained a partial response in 3 cases with incomplete excision. No unfavourable outcomes have been observed. Genetic screening is a peculiar aspect of this neoplasm, and biological studies (screening for Von Hippel-Lindau disease, VHL gene, RET, SDHD and SDHB genes) are included in the evaluation of patients with pheochromocytoma registered in the TREP project.

**Renal Cell Carcinoma.** Renal cell carcinoma (RCC) is a malignancy arising from the epithelial cells of the renal tubule; it is typical of adult age and the therapeutic recommendations for children follow the criteria derived from the adult experience, though there is some speculation as to whether RCC in children differs from its adult counterpart.

In pediatric series, the mean age at presentation is over 10 years (in contrast with 3-4 years of age for Wilms’ tumor), so the possibility of RCC should be considered in cases of a renal mass occurring in older children. The TREP project includes diagnostic and therapeutic recommendations and biological studies (on the VHL gene). The proposed risk-adapted treatment program stems from experience with adults. Radical nephrectomy is the mainstay of treatment (and the only really effective therapy); regional lymph node biopsy (or lymphadenectomy in the event of clinical involvement) is recommended. Surgery alone is curative for stage I patients (complete excision of the disease), whereas immunotherapy IL-2 could be considered in case of local invasiveness and metastases. At present 34 patients have been registered in the TREP study and 27 are alive: all the 24 cases undergone complete surgery are in complete remission and 19/24 did not receive any further therapy.

**Pleuropulmonary Blastoma** Pleuropulmonary blastoma (PPB) is a highly aggressive pulmonary malignancy that occurs in young children as a true dysembryogenic neoplasm of the pulmonary mesenchyma. The association with pre-existing
cystadenomatoid malformations (CCAM) has been demonstrated: many reports suggest to operate on these lung malformations within the first year of age to avoid the neoplastic risk. PPB can be subclassified as purely cystic (type 1), cystic and solid (type 2) and purely solid (type 3). The optimal treatment strategy for PPB patients is still not clear, due to the paucity of reported series. Despite the introduction of multimodal therapy (surgery, chemotherapy, and occasionally radiotherapy), the prognosis is uncertain because of loco-regional invasiveness (pleural and mediastinal invasion) and a tendency for metastatic spread. Priest et al. reported a better 5-year OS for type I than for types II and III. Complete surgery remains the keystone of treatment: unfortunately usually it is not feasible at diagnosis due to dimensions of the mass and its extrapulmonary involvement. In the TREP strategy VAIA chemotherapy regimen (vincristine, ifosfamide, doxorubicin, actinomycin) usually adopted for soft tissue sarcomas was chosen due to some satisfactory responses obtained in previous cases; radiotherapy could be used in selected cases. An analysis by Indolfi et al. on 22 cases (5 with pre-existing congenital cysts) showed that the achievement of total resection of the tumor at any time of treatment (primary surgery or delayed surgery) resulted in a significantly better prognosis, whereas extrapulmonary involvement at diagnosis resulted in a significantly worse prognosis. Estimated 5-year event-free and overall survival rates were 44 and 49% for all patients, respectively.

Non Germ Cell Gonadal Tumors are a heterogeneous group of tumors which includes sex cord stromal tumors (SCST) either in males and females and ovarian epithelial tumors, with different clinical features and biologic behaviour. The ovarian SCST account for 8-10% of all ovarian cancers. The granulosa-theca cell tumors represent the largest subgroup (7-8%): although they can occur in women of any age, the juvenile granulosa cells tumor (JGCT) is typical of the first two decades of life. JGCT is characterized by isosexual precocity or virilization and has a less aggressive behaviour than that of adult variety. Another important but less frequent subgroup is constituted by Sertoli-Leydig cell tumors, which represent 1-2% of all ovarian tumors with more frequent malignant course. Most of them produce male hormones, which may be used as clinical markers. The histological retiform subtype is linked to an aggressive behaviour. Also testicular SCST are very rare in young patients (about 4-5% of all testicular tumors). They may be found in all age groups, with 2 peaks that occur within few months following birth and around puberty respectively. Leydig cell tumors is the most common histotype; characterized by symptoms of isosexual pseudoprecocity due to androgenic hormone production. Sertoli cell tumors represent another subgroup that can be associated with Peutz-Jeghers syndrome and Carney complex. Granulosa cells tumors may arise also in testicular tissue, and sometimes granulosa cells are intermixed to constitute a mixed sex cord stromal category. All these forms may present with a testicular mass and with or without hormonal manifestations such as isosexual pseudoprecocity or estrogenic manifestations, and generally have a benign behaviour. The primary management is eminently surgical: the excision of ovarian masses is usually feasible at diagnosis and an accurate intraperitoneal staging is always required. For testicular SCST, whose behaviour is generally less aggressive a conservative surgery is recommended in selected cases. The TREP indications for chemotherapy for SCST should follow the guidelines of pediatric malignant germ cell tumors and for epithelial ovarian tumors those adopted for adult ovarian malignancies. Among 19 ovarian SCST observed between January 2000 and December 2008 18 were cured and 15/18 with surgery alone, while all 10 testicular SCST were cured, 5/10 with a conservative surgery.

Thyroid Carcinoma. Nodules of the thyroid gland are rather uncommon in pediatric age, however they have a relatively high association with cancer. The papillary and follicular carcinomas are the most important forms of carcinoma, being the well differentiated papillary carcinomas the more common histotype. Typically it presents as either a thyroid mass, or enlarged cervical nodes and pediatric surgeons may be called on to evaluate a suspected mass. The diagnostic work up and the therapeuthic approach to these tumors generally requires specific skills: surgery has the most important role however the surgical options include some
different approaches (thyroid lobectomy, near-total thyroidectomy and total thyroidectomy) on the basis of the clinical investigations and the experience of the surgeon. It has been clearly demonstrated that pediatric patients with this tumor have an excellent prognosis despite advanced disease at diagnosis. Medullary Thyroid Carcinoma is very rare in children: it may be sporadic (identified through basal calcitonin test) or may occur as a manifestation of the hereditary Multiple Endocrine Neoplasia type 2 syndrome (MEN2). Surgery is the only curative method of treatment both in sporadic and inherited MTC. It includes total thyroidectomy with elective lymph node dissection.

Until now 104 cases with thyroid carcinoma were registered in the TREP project (about 95% with differentiated carcinomas): surgery is performed only in a few pediatric surgery centers. Also within the TREP group, there has been a debate on the most adequate treatment for well-differentiated thyroid carcinoma. Two analysis concerning also patients observed before the TREP Study started, confirmed an excellent outcome of patients irrespective of type of surgical strategy.

An adequate risk stratification is needed to identify patients requiring total thyroidectomy (with or without lymphadenectomy) and those who could be treated with conservative surgery.

**Conclusive Remarks**

The active involvement of Pediatric Surgeons in the TREP project has contributed to improve the results. A favourable factor to increase the recruitment of cases has been to serve other clinician and support their decisions. We believe that surgery has an important role in the initial diagnostic work up and along the treatment for most rare solid tumors. Also the achievement of a good cooperation with other specialists has been an important objective.

The variety of tumors requires collaboration among surgeons with different expertise (i.e. a surgeon may be skilled in thyroid tumors and another in thoracic tumors another in skin cancer). Therefore the adoption of working groups for each tumor type in a collaborative network has been a key element of success. Moreover a strict collaboration with adult surgeons is needed when tumors very frequent in adults are encountered (i.e. breast cancer, colon cancer).

The next effort to improve our knowledge and clinical approach to rare tumors should be an international cooperation with the aim to collect cases from a larger number of children and to develop a cooperative group of pediatric oncologists, pediatric surgeons and other specialists dedicated to children with these tumors.

**Summary**

a) Every pediatric surgeon may encounter a rare tumor during his activity. Pediatric surgeons should be aware of the existence of these tumors and should know the correct diagnostic work-up and treatment.

b) Several solid rare tumors require surgical therapy alone, which may be curative if correctly performed. For other rare tumors, which need a multidisciplinary therapy, pediatric surgeons must know the role and the timing of surgical therapy; collaboration with other specialists is required.

c) Some tumors are exceptional in pediatric age, whereas they are very common in adults (colon cancer, breast cancer): in these cases the task for pediatric surgeons is to recognize the disease, while the surgical treatment should be performed together with experts on adult cancer.

d) A typical aspect of rare tumors concerns the variety of histotypes in different sites: this particular characteristic requires surgeons with different expertise who should be able to collaborate together in a network. The Italian TREP Project has demonstrated the feasibility of such a framework, which may be a model for developing international collaborations.

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Infections in Children with Acute Myeloid Leukemia: Lessons Learned from International Co-operative Group Trials and Low Income Countries

Lillian Sung

Infection is an important complication and cause of death in children receiving highly myelosuppressive chemotherapy for treatment of acute myeloid leukemia (AML). Therapy for AML targets the myeloid cell lineage, producing profound and prolonged neutropenia and it is therefore one of the most intensive chemotherapeutic regimens used in pediatric oncology. Consequently, many children experience one or more infectious complications during AML treatment.[1,2] Infections are important to study in pediatric cancer because they continue to contribute to morbidity and mortality, require considerable health resources to prevent and treat, and they affect quality of life for children and their families. In AML, infections are particularly important because of the relatively high rate of infection-related mortality, costs associated with newer drugs to treat specific infections such as anti-fungals, and resultant common practice of mandatory hospitalization prior to evidence of count recovery.

Insights into risk factors and preventative or treatment strategies for infection outcomes in this population may be derived from co-operative group or national clinical trial data. The purpose of this presentation is to illustrate how we have gained such insights through data available from the North American consortium, the Children’s Oncology Group (COG), and the German based consortium, Berlin-Frankfurt-Muenster (BFM). Additional insights also have been gained through descriptive analyses in the low income country of El Salvador.

In the COG setting, there are two phase III AML trials in which infections were carefully collected and analyzed. The more recently conducted study was CCG 2961.[3] Therapy consisted of four phases: (1) Induction (idarubicin, daunomycin, cytarabine, thioguanine, etoposide, and dexamethasone (IdaDCTER)); (2) Consolidation, (randomization between a second IdaDCTER versus fludarabine, cytosine arabinoside, and idarubicin); (3) Intensification (cytosine arabinoside and L-asparaginase or allogeneic stem cell transplantation (SCT) for those patients with a matched related donor); and (4) Immune Modulation, (randomization to interleukin-2 or none in patients without donors after phase 3). We examined microbiologically defined infections and infection-related mortality in 492 children with AML enrolled on this study.[2] The median age was 9.6 years (range 0.005, 21.0) years. Of the 492 children, 48 were overweight (10%) and 150 (30%) were underweight. During phase 1, 33 (7%) died. Over 60% of children experienced microbiologically documented infections in each of the first three phases of chemotherapy. Infections with Gram-positive cocci predominated as the etiology of microbiologically documented infections. More specifically, coagulase-negative Staphylococcus and viridans group Streptococcus were the most common causes of at least one infection during each phase of therapy, with 18%, 17% and 19% of patients having at least one positive culture with coagulase-negative Staphylococcus, and 10%, 27% and 20% of patients having at least one positive culture with viridans group Streptococcus, in phases 1, 2, and 3 respectively. Pseudomonas species, Escherichiae coli and Klebsiella species were the most common Gram-negative organisms. Fungi also were a prominent cause of infections, occurring in 18%, 21% and 14% of subjects in phases 1, 2 and 3 respectively. Four to 10% of patients had at least one infection with Candida species. There were 58 infectious deaths; cumulative incidence of infection related mortality during chemotherapy (and excluding SCT) was 11 ± 2%. Thirty-one percent of infectious deaths were associated with Aspergillus, 25.9% with Candida, and 15.5% with viridans group
streptococci. Age over sixteen years (hazard ratio (HR) 3.32, 95% confidence interval (CI) 1.87, 5.89; \( P < .001 \)), non-white ethnicity (HR 1.85, 95% CI 1.10, 3.09; \( P = .02 \)) and underweight status (HR 3.06, 95% CI 1.51, 6.22; \( P = .002 \)) were associated with infection-related mortality while size of the treating institution was not. Thus, age, ethnicity and body mass index were important contributors to infection-related mortality. Fungi and Gram positive cocci were the most common organisms associated with infectious deaths and in particular, \( \textit{Aspergillus} \) species was the largest contributor to infectious deaths.

The second COG AML study was CCG 2891.[4] In this study, during Phase 1 (induction), patients were randomized to intensive or standard timing. In Phase 2 (consolidation), those with a family donor were allocated SCT; the remainder was randomized to autologous SCT or chemotherapy. We compared infections between different treatments on an intent-to-treat basis. During Phase 1, intensive timing was associated with more bacterial (57.7% vs. 39.4%, \( P < .001 \)), fungal (27.4% vs. 9.9%, \( P < .001 \)) and viral (14.0% vs. 3.9%, \( P < .001 \)) infections compared to standard timing. We noted either significant or non-significant tendencies for more infections during intensive timing with most Gram positive and negative pathogens. In particular, there was a significance increase in viridans group streptococcal infections associated with intensive timing induction. In contrast, infections with coagulase negative staphylococci and \( \textit{Staphylococcus aureus} \) occurred at a similar frequency in the intensive and standard timing groups. Fungal infections also were more common in intensive timing for both yeasts (19.0% vs. 8.4%, \( P < .001 \)) and molds (11.7% vs. 1.5%, \( P < .001 \)). Finally, viral infections occurred more frequently during intensive timing (14.0% vs. 3.9%, \( P < .001 \)). During Phase 2, chemotherapy was associated with more bacterial (56.5% vs. 40.1%, \( P = .005 \)), but similar fungal (9.5% vs. 6.1%, \( P = 1.000 \)) and viral (4.2% vs. 12.9%, \( P = .728 \)) infections compared with allogeneic SCT. No differences between chemotherapy and autologous SCT infections were seen. Fatal infections were more common during intensive compared with standard timing induction (5.5% vs. 0.9%; \( P = .004 \)). Infectious deaths were similar between chemotherapy, autologous SCT and allogeneic SCT. Therefore, we found that on CCG 2891, a phase III pediatric AML trial, more bacterial, fungal and viral infections were associated with intensive timing compared with standard timing and more infectious deaths were associated with intensive timing. However, this increase did not occur uniformly and infections with \( \textit{Staphylococcus} \) species occurred at a similar frequency. In contrast to our expected finding, more bacterial infections and more viridans group streptococcal bacteremia were associated with Phase 2 chemotherapy compared with allogeneic SCT. We also found that Gram negative organisms and molds continue to contribute to fatal infections in pediatric AML.

In trying to understanding risk factors for infection, it may be useful to compare outcomes between different AML co-operative groups such as trials conducted by COG and the Berlin-Frankfurt-Muenster (BFM) group.[5] Bacteria are the most common pathogens of microbiologically documented infection in pediatric AML. Cooperative groups have consistently noted the predominance of Gram positive infections in pediatric AML and the high risk of fulminant viridans group streptococcal bacteremia. In BFM-93, 203/252 (81%) of isolates causing bloodstream infections were Gram positive organisms and viridans group streptococci comprised 56/252 (22%) of all isolates.[1] In CCG 2961, 191/297 (64%) of microbiologically documented infection in induction were Gram positive organism, with 51/297 (17%) due to viridans group streptococci.[2] While Gram negative infections were less common, they continue to be an important contributor to severe infections. The two most common Gram negative bacteria on both BFM-93 and CCG 2961 were \( \textit{Escherichiae coli} \) and \( \textit{Pseudomonas aeruginosa} \), suggesting that anti-pseudomonal coverage remains an important component of empiric antibacterial therapy for febrile neutropenia in this population. The other consistent finding is the polymicrobial nature of invasive infections in pediatric AML. In CCG 2961, over 60% of infectious deaths were associated with more than one microorganism and 27% of children in induction experienced infections with more than one pathogen. Finally, in both CCG 2961 and BFM-93, invasive fungal infections were a major cause of morbidity and mortality in pediatric AML.
Infections due to Candida and Aspergillus were the most common fungal pathogens. Importantly, in both CCG 2961 and BFM-93, Aspergillus was the most common cause of infectious mortality, thus highlighting the problem of invasive fungal infections in pediatric AML. Therefore, in putting these observations together with respect to infections on CCG 2961 and BFM-93, the etiology and implications of invasive infections appear to be quite similar within these two co-operative groups in spite of very different treatment protocols and differences in health care delivery, geography, ethnicity and supportive care as discussed below.

Another issue that impedes understanding of infection outcomes in clinical practice and clinical trials is substantial differences in supportive care practices between centers and trials. For example, we had believed there would be substantial variation in local practice patterns regarding mandatory hospitalization for children with AML prior to bone marrow recovery, and approaches to prophylactic colony-stimulating factors, antibiotics and antifungals; these practices may impact on infections, costs and quality of life. In order to explore this issue, we surveyed infection-related supportive care practices among centers participating COG and BFM and determined whether these differed by group. In total, 216 COG institutions and 55 BFM institutions were included. Since COG includes centers from the United States, Canada, Switzerland, Australia and New Zealand, six countries were included in this study. The overall response rate was 83.8% (227/271). The response rate was 180/216 (83.3%) for COG and 47/55 (85.5%) for BFM. Most institutions had less than 5 new diagnoses of AML per year (158/227, 69.6%). Overall, most centers did not routinely use antibacterial prophylaxis but did routinely use antifungal prophylaxis. BFM centers were significantly more likely to routinely use antibacterial prophylaxis (24/180, 13.3%) compared with COG centers (15/46, 32.6%; P<.0001). More specifically, BFM centers were more likely to use penicillin prophylaxis (6/180, 3.3%) compared to COG centers (10/180, 21.3%; P<.0001). Similarly, BFM centers were significantly more likely to use routine antifungal prophylaxis (42/46, 91.3%) compared to COG centers (137/178, 76.5%; P=.03). BFM centers were more aggressive regarding discharge home prior to evidence of bone marrow recovery, with 21/42 (50.0%) discharging children home early compared to 36/75 (20.6%) of COG centers. The results of this study are important as they illustrate how different countries and study groups have systematically different practices. Interestingly, despite these different practices (for example, different rates of antibacterial and antifungal prophylaxis), there are many similarities in the nature of infections on COG and BFM trials as described above although comparisons of infection outcomes have only been indirect to this point. If infection outcomes are indeed similar in spite of different supportive care practices, then the currently employed supportive care practices may not be effective in preventing invasive infections. Second, we may be able to use this variability in practice to determine the real-world effectiveness of supportive care interventions such as antibiotic prophylaxis. In order to conduct such an analysis, harmonization of definitions and endpoints between study groups is important and we have begun such efforts between COG and BFM. Finally, this data may be used to design future interventional trials by identifiable the most likely acceptable standard arm for such a trial.

More insight into infection and treatment-related mortality may be gained by examining infections in pediatric AML within a completely different context such as low income countries. Survival rates among children with leukemia in low income countries are lower than in high income countries. Previous studies have attributed this in part to higher treatment and infection-related mortality. In order to better describe this problem, we examined children with AML diagnosed and treated in El Salvador, a low income country in Central America, from January 2000 to June 2007. [6] Data were collected prospectively by trained data managers; no patients were excluded. During the study time frame, patients with AML were treated according to protocols based on BFM-AML93, and NOPHO-AML 93. 78 patients with AML were included. The two-year cumulative incidence of treatment-related mortality was 35.4±6.4% and 25/47 (53.2%) of deaths were attributable to the toxicity of treatment. Of the 25 toxic deaths, 14 (60.9%) were due to infection, 7 (30.4%) were due to bleeding, and 2 (8.7%) were due to organ failure. Among children with AML, biologic, socioeconomic and
nutritional variables were not associated with treatment-related mortality. Thus, in the low income country setting of El Salvador, infection-related mortality also is a prominent cause of treatment failure in AML. However, unlike the high income country setting, age and malnutrition do not appear to be risk factors for infectious deaths.

In summary, infections and infection-related mortality are a major problem for children with AML in all settings irrespective of systematic differences in chemotherapy protocols, supportive care practices, resources and healthcare delivery. Analyses from all of these settings have shed insight into risk factors for infections and further research focused at reducing infection outcomes should be a priority globally.

References
Building evidence based Practice in Pediatric Oncology

N. Kline

Abstract
Despite an aggressive research agenda, the majority of findings are from research are not integrated into practice. Without current best evidence, practice becomes rapidly out-of-date to the detriment of patients. Medical and nursing practices based on the best available evidence are more likely to achieve quality patient outcomes. In a busy hospital environment policies and procedures that drive patient care frequently become routine and as technologies rapidly change and new treatments constantly emerge, medical and nursing professionals must think critically about their practice. An institutional infrastructure to support the EBP process is useful to constantly support current practice and explore clinical questions. At times, the most sophisticated practices or treatments are not feasible or obtainable and at that point it is important to choose the current best evidence that can be supported in the practice setting.

Introduction
Evidence-based practice (EBP) is the conscientious use of current best evidence, while also considering individual patient differences and resource constraints in providing patient care. Using a standardized EBP approach may help to provide consistency of evidence reviews, synthesis and recommendations for practice. An EBP culture is a practice environment that values high-level evidence based care and maintains partnerships between disciplines to support patient care.

The EBP Process
A five-step approach is often used in EBP reviews; 1) framing a clinical question, 2) searching for evidence, 3) analyzing the evidence, 4) synthesizing the evidence, and 5) making practice recommendations.

Framing a clinical question
Clinical questions may arise from a variety of sources, including information obtained during a professional conference, anecdotal observations during patient care, discussions with colleagues. A brief description of the background and significance of the question is important as well as identifying objectives to guide the search for the evidence. The clinical question should be focused and not attempt to cover excessive clinical content.

Searching the evidence
Appropriate key words should be identified and multiple databases should be searched, if possible. Literature search engines (e.g., PubMed, EMBASE, CINAHL), evidence-based web sites (e.g., Evidence Matters, Johanna Briggs Institute), organizational web sites (e.g., Oncology Nursing Society) are excellent sources of information and current evidence. The Cochrane Collection is composed of a group of over 15,000 individuals who perform systematic reviews of healthcare interventions and also non-randomized, observational studies. The goals include providing access to evidence-based reviews and keeping practice up to date. Ideally, a medical librarian can assist with the process, but if not available it is often helpful to ask a colleague to review the identified key words. If the research literature does not yield any results, the best available evidence may be in the form of professional organization guidelines or expert opinion.

Evidence is ranked on a variety of scales. The ranking simply provides assignment of a “level” to the evidence. It does not provide “strength” of evidence that comes later in the form of appraisal of the evidence (Tables 1 and 2).
Table 1: Evidence Hierarchy

<table>
<thead>
<tr>
<th>Level</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Level I</td>
<td>A systematic review or meta-analysis of all relevant randomized control trials (RCT) or evidence-based clinical practice guidelines based on systematic reviews of RCTs</td>
</tr>
<tr>
<td>Level II</td>
<td>RCT</td>
</tr>
<tr>
<td>Level III</td>
<td>Controlled trials without randomization</td>
</tr>
<tr>
<td>Level IV</td>
<td>Case control and cohort studies</td>
</tr>
<tr>
<td>Level V</td>
<td>Systematic reviews of descriptive and qualitative studies</td>
</tr>
<tr>
<td>Level VI</td>
<td>A descriptive or qualitative study</td>
</tr>
<tr>
<td>Level VII</td>
<td>Opinion of authorities and/or reports of expert committees</td>
</tr>
</tbody>
</table>

Table 2: Centers for Disease Control (CDC)/ Healthcare Infection Control Practices Advisory Committee (HICPAC) System for Categorizing Recommendations

<table>
<thead>
<tr>
<th>Level</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Category IA</td>
<td>Strongly recommended for implementation and strongly supported by well-designed experimental, clinical, or epidemiologic studies</td>
</tr>
<tr>
<td>Category IB</td>
<td>Strongly recommended for implementation and supported by some experimental, clinical, or epidemiologic studies, and a strong theoretical rationale.</td>
</tr>
<tr>
<td>Category IC</td>
<td>Required by state or federal regulations, rules, or standards.</td>
</tr>
<tr>
<td>Category II</td>
<td>Suggested for implementation and supported by suggestive clinical or epidemiologic studies or a theoretical rationale.</td>
</tr>
<tr>
<td>Unresolved issue</td>
<td>Represents an unresolved issue for which evidence is insufficient or no consensus regarding efficacy exists</td>
</tr>
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</table>
Nephrogenic Rests and Nephroblastomatosis

Elizabeth J. Perlman

Abstract
Nephrogenic rests represent precursor lesions to nephroblastoma. They are classified into perilobar (PLNR) and intralobar (ILNR) types. PLNRs are well-circumscribed, located at the edge of the lobule, and composed of epithelial and blastemal cells. PLNRs that present particular diagnostic challenges include diffuse and adenomatous types. ILNRs are located deep in the kidney, intermingle among the normal renal parenchyma, and commonly show stromal differentiation. ILNRs may be quite large and their distinction from Wilms tumor is difficult. Knowledge of the genetic events that underlie the development of nephrogenic rests is limited. However, ILNRs are most frequently associated with WT1 mutation, and ILNRs themselves commonly show WT1 mutation. The development of Wilms tumor within an ILNR may be associated with CTNNB1 mutation. PLNRs, in contrast, show imprinting changes 11p15 and gain of expression of IGF2. Nephrogenic rests are often best diagnosed and followed by radiology rather than pathologic assessment. Chemotherapy is often utilized in the therapy of nephrogenic rests, particularly those that are large or that are located deep in the kidney.

Introduction
Nephrogenic rests are defined as an abnormally persistent foci of embryonal cells beyond 36 weeks within developmentally normal kidneys. Nephroblastomatosis refers to multiple or diffuse nephrogenic rests. Nephrogenic rests represent precursors of nephroblastoma, and are encountered in approximately 25% of patients with nephroblastoma. Nephrogenic rests are classified into perilobar (PLNR) and intralobar (ILNR) types. PLNR is found in approximately 1 percent of infant autopsies whereas ILNR is found in only 0.1 percent. Both types of nephrogenic rests are associated with syndromes carrying a high risk for nephroblastoma. Patients with any type of nephrogenic rest identified within a kidney removed for nephroblastoma should be considered at increased risk for tumor formation in the remaining kidney; the risk is the greatest in patients less than 12 months of age.

Pathogenesis
The genetic events involved in the development of a nephrogenic rest, and in the development of a Wilms tumor within a nephrogenic rest, have been little studied due to the difficulty in obtaining nephrogenic rest tissue. Four studies of particular interest are summarized below:

- Fukuzawa et al. examined the nephrogenic rests of two patients whose Wilms tumors had both WT1 and CTNNB1 mutations. The ILNRs in both cases showed WT1 mutation but not CTNNB1 mutation. This suggests that CTNNB1 mutation is a later event in Wilms tumorigenesis.
- Vuononvirta et al analyzed 50 PLNRs and 25 corresponding WT. 22/50 PLNRs showed no copy number changes; 8/50 PLNRs showed a single whole chromosome alterations; 20/50 PLNRs showed multiple gains/losses. The most frequent aberrations included loss of 1p, gain of 18, 13, 12. Of interest, the majority of rests that were tested were classified as sclerosing or regressive, and the majority of PLNRs that showed genetic changes were of these subtypes and not of the hyperplastic subtypes. The vast majority of PLNRs showed loss of imprinting at 11p15 with increased expression of IGF2.
- Park et al looked at 19 cases of WT that contained nephrogenic rests and found WT1 mutation in 2/19. One mutation was found in both an ILNR and adjacent Wilms tumor, but not in the patient’s normal kidney. The second WT1 mutation was identified in both a Wilms...
tumor and adjacent lesion classified as hyperplastic PLNR in a patient with BWS syndrome.  

- Charles et al performed LOH analysis on Wilms tumors and their associated rests.  

For 12 patients with associated PLNRs, only one tumor and none of the PLNRs demonstrated LOH for either 11p13 or 11p15. For 14 patients with ILNRs, two demonstrated LOH in both rest and tumor for 11p13 and 3 demonstrated LOH for 11p15 in both rest and Wilms tumor.

Taken together, these studies suggest that at least some ILNRs are clonally derived from an early renal stem cell. Inactivation of WT1 appears to be an early genetic event that can lead to the formation of ILNR; additional genetic events (perhaps including CTNNB1 mutation) may then lead to the development of WT. PLNRs are reliably characterized by imprinting abnormalities at 11p15 associated with increased expression of IGF2. It is likely that many PLNRs have not progressed as far along the pathogenetic pathway toward Wilms tumors as ILNRs, with fewer genetic changes. It has been proposed that PLNRs may arise after loss of imprinting rather than LOH at 11p.  

**Perilobar Nephrogenic Rests**

PLNRs are characterized by sharp circumscription and a location at the periphery of the lobule. They are most commonly located on the surface of the kidney, but may also be seen deep within the renal parenchyma along the edges of the lobule where the cortex follows the columns of Bertin. PLNRs may be seen at different stages of their development, resulting in different morphologies. Dormant or incipient rests are small, microscopic lesions with no evidence of proliferation, maturation, or involution. These rests usually contain well-formed tubular structures lined by a single layer of low cuboidal basophilic epithelium containing few or no mitotic figures. When PLNRs begin proliferating, they become larger, yet maintain an oval shape. Dormant rests or incipient rests may regress, or may become more actively proliferative. Such hyperplastic nephrogenic rests appear microscopically to be composed of poorly differentiated or undifferentiated epithelial and blastemal cells. Hyperplastic rests may be indistinguishable from Wilms tumor cytologically. The most distinctive features of hyperplastic PLNRs are a tendency to preserve the original shape of the rest and a direct interface with the adjacent renal parenchyma. A proliferating nephrogenic rest may then have one of several fates. Most commonly, the rest will regress, resulting in peritubular scarring and decreased proliferation. Following regression, islands of cells within the rest may have continued proliferation that may wax and wane over time. The end stage of a sclerosing rest is termed an obsolescent rest. These lesions are composed entirely of collagenous stroma and may be difficult to identify with certainty in the absence of other rests.

Included in Beckwith's widely accepted classification of nephrogenic rests is a rare histologic subtype known as adenomatous nephrogenic rest. These foci are characterized by well-circumscribed nodules developing within existing nephrogenic rests that are composed of differentiated epithelial cells containing moderate eosinophilic cytoplasm, rather bland nuclei, and a paucity of mitotic figures. Architectural features that may be seen include tubular and/or papillary formations. The nodules are sharply circumscribed, and often compress the adjacent rest tissue. Beckwith initially considered it possible, if not likely, that such lesions represent neoplastic clones developing within the underlying nephrogenic rests, however increased opportunities to observe the occurrence and outcome of adenomatous nephrogenic rests shed some doubt on this. First, adenomatous nephrogenic rests may be identified at any point in the course of nephrogenic rests, including pre-therapy biopsy or nephrectomy specimens. Second, when they are identified, adenomatous nephrogenic rests are virtually always numerous, and are scattered about the specimen received with sizes varying from one millimeter to 3-4cm, but all showing the same histologic appearance. This suggests that the development of adenomatous nephrogenic rest is due to the triggering of a particular differentiation pathway rather than a clonal evolution. Third, the clinical behavior of these lesions has not impacted the survival of patients with nephroblastomatosis. None have shown metastasis, invasion, or other clinical difficulties secondary to adenomatous nephrogenic rests. Therefore, the biologic
potential of the vast majority of these lesions is quite low.

There are a number of features that make the distinction between Wilms tumor and adenomatous rest difficult in some cases. First, a striking feature most commonly observed in post-therapy specimens is the presence of a peripheral rim of collagen separating the adenomatous rest from the adjacent sclerosing nephrogenic rest. This is indistinguishable from a fibrous capsule, a feature that currently comprises one of the more useful diagnostic criteria of the development of Wilms tumor within a PLNR. Second, these lesions may rarely be large, although a single lesion rarely exceeds 3cm. Lastly, within the files of the JB Beckwith Developmental Renal Tumors, there have been very rare cases seen in which a mass with similar histologic features was associated with lymph node metastasis. None of these arose within the setting of perilobar nephroblastomatosis; one arose within an intralobar nephrogenic rest. Instead, these have been de novo tumors that likely represent variants of epithelial Wilms tumor or papillary renal cell carcinoma.

**Diffuse perilobar nephroblastomatosis:**

In rare instances, most or all of the cortical surface of one or both kidneys may be involved by a continuous band of perilobar nephroblastic tissue in a condition classified as *diffuse perilobar nephroblastomatosis (DHPLN)*. This results in massive renal enlargement often mistaken for Wilms tumor. Children with DHPLN commonly present at 1-3 years of age with unilateral or bilateral renal masses. The renal enlargement may be massive, with weights up to 1500g. In children with DHPLN who have received radiographic studies prior to the onset of their disease, normal or slightly enlarged kidneys with no mass lesions were present at birth. This suggests that these children are born with a small rim of residual nephroblastic tissue which then, by some unknown trigger, becomes hyperplastic.

The gross appearance of DHPLN is often quite striking, with a rind-like band of tan soft tissue replacing the renal cortex. This band of nephroblastic tissue demonstrates a somewhat irregular and scalloped contour, and a direct interface with the kidney without a fibrous capsule. Histologically, DHPLN is composed of the primitive epithelial and blastemal components seen in hyperplastic perilobar rests, often arranged in a confluence of nodules corresponding to the renal lobes. At the periphery of the nodules, compressed and less proliferative nephroblastic tubules are often found and merge directly with normal renal parenchyma. The distinctiveness of this lesion from nephroblastoma is evident radiographically by the diffuseness and homogeneity of the cortical process, with similar or slightly lower echogenicity and signal intensity when compared to the renal cortex. The centrally located residual parenchyma often demonstrates a characteristic scalloped or speculated appearance after contrast enhancement. The diagnosis of nephroblastoma is commonly erroneously made unless the distinctive radiographic appearance is noted. As with all hyperplastic PLNRs, the fate of DHPLN is either regression or the development of nephroblastoma. Because of the tremendous burden of proliferating nephroblastic cells, the risk for the development of a nephroblastoma is extraordinarily high in patients with DHPLN. For this reason, the chemotherapeutic regimen recommended for low stage, favorable histology nephroblastoma is commonly utilized in the treatment of patients with DHPLN, particularly when the lesions are bilateral. Approximately half of patients with DHPLN who are provided chemotherapy have complete resolution of the process and never develop tumors subsequently. The remaining patients have a spectrum of waxing and waning growth, both in the development and size of their rests and in the development of nephroblastoma over a course of 5-10 years. Therefore, the most critical determinant of long-term survival of the patient is the utilization of a treatment regimen that preserves renal parenchyma. Lastly, patients with DHPLN have a 32 percent risk of developing anaplasia, likely simply due to the increased number of tumors per patient. Therefore, their tumors must be carefully watched and monitored for responsiveness to therapy.

**Development of Wilms tumor within a perilobar nephrogenic rest**

When a clonal expansion develops within a perilobar nephrogenic rest, this results in a nephroblastoma. A nephroblastoma developing
within a PLNR is recognized by its propensity for spherical expansile growth and a peritumoral fibrous capsule separating the neoplasm from the adjacent rest and normal kidney. The cytology of the nephroblastoma is often indistinguishable from hyperplastic PLNR, and is characterized by blastemal and poorly differentiated epithelial or stromal components. The distinction between nephrogenic rest and nephroblastoma can be made with confidence pathologically only when the shape of the lesion and the interface between the lesion and the surrounding kidney is included in the surgical excision. Therefore, a diagnosis of Wilms tumor or PLNR by needle biopsy or fine needle aspiration is often not possible.

**Intralobar Nephrogenic Rests**

ILNRs are typically located in the central areas of the renal lobe, are poorly circumscribed, and interdigitate with the normal renal parenchyma. ILNRs commonly contain stromal elements as well as epithelial tubules, and may contain adipose tissue whereas PLNRs lack a stromal component. Like PLNRs, ILNRs may undergo hyperplasia, and this hyperplasia may involve stromal, epithelial and/or blastemal elements. For these reasons, the distinction between a large hyperplastic ILNR and nephroblastoma may be quite difficult. Clues to the correct diagnosis include the intermingling interface with the normal kidney characteristic of ILNR, and the histologic components of the lesion itself. For example, with few exceptions, fat is commonly found in ILNR but is uncommon in nephroblastoma; conversely skeletal muscle differentiation is uncommonly found in ILNR but common in nephroblastoma. The ability to be confident that a Wilms tumor has not developed within a hyperplastic ILNR in inversely proportional to its size. For this reason, it has been our practice to provide a diagnosis of Wilms tumor when full confidence is not possible.

While PLNRs are always located in the kidney, ILNRs may occasionally be found in the soft tissue of the renal sinus, and may also form a suburothelial pad of nephroblastic tissue in the renal pelvis. Care needs to be taken to not over-stage such lesions. Rare examples have presented to the NWTSG pathology center as cystic abdominal masses which on review are ILNRs that have developed within a duplicated ureter.

While the majority of PLNRs undergo regression and sclerosis without the development of a nephroblastoma, the risk of developing a nephroblastoma is considerably higher within an ILNR, and the frequency of uneventful regression is thought to be quite low, although data specifically addressing this is limited. The nephroblastoma developing with an ILNR is often, but not always, separated from the underlying rest by a peritumoral fibrous capsule. Many nephroblastomas associated with ILNRs have a greater degree of heterogeneity that those arising in PLNRs, and often contain abundant stromal and heterologous elements such as differentiated skeletal muscle cells and cartilage. Such nephroblastomas have been classified as "central" in contrast to the "peripheral" nephroblastomas associated with PLNRs that demonstrate predominantly epithelial and blastemal elements. ILNR-associated nephroblastomas are most common in young infants, and such cases have a very high risk of developing contralateral lesions in the future.

**Differential Diagnosis.** Rare entities that may be mistaken for nephrogenic rests include the dysplastic medullary ray nodules that are most commonly seen in association with Beckwith-Wiedemann syndrome. These are larger and more disorganized than the medullary ray nodules that may be seen in normal infant kidneys. Foci of embryonal hyperplasia found in end-stage kidneys and in patients with dysplastic kidneys may also be mistaken for nephrogenic rests.

**References**


Epigenetic Maintenance of Stemness in Pediatric Malignancy

Stefan Burdach and Günther HS Richter

Abstract
A shift of the focus in cancer research from genetic to epigenetic mechanisms can be observed during the last five years. Chromatin modifications are increasingly recognized as a key mechanism. The histone methyl-transferase Enhancer of Zeste, Drosophila, Homolog 2 (EZH2) is the enzymatic subunit of the polycomb PRC2 complex and methylates histone H3K27, thereby, mediating gene silencing. EZH2 is over-expressed in a variety of tumor tissue including breast and prostate. Ewing Tumors (ET), are highly malignant tumors that are molecularly defined by ews/ets translocations, with fli1 being the ets family member most frequently involved. We found EWS-FLI1 bound to the EZH2 promoter in vivo. Other components of the PRC2 complex, like EED or SUZ12 were not deregulated in ET. Down-regulation of EZH2 by RNA interference suppressed tumor development and metastasis in vivo and revealed an EZH2-maintained undifferentiated, reversible phenotype in ET. EZH2 suppression resulted in a generalized loss of H3K27me3 as well as an increase in H3 acetylation. Gene specific analysis of H3K27me3 verified such genes that had specifically lost H3K27me3 upon EZH2 silencing, suggesting that stemness features are preserved via epigenetic mechanisms and that the genetic EWS-FLI1 translocation is intimately linked to global and gene specific epigenetic alterations in ET biology.

Introduction
By definition, a climax marks the begin of decline. With regard to the genetic paradigm, the sequencing of the human genome has been no exception to that rule. The amazing finding of only approximately 20,000 – 25,000 genes in the humane genome (comparable with the number of genes in the worm genome of C. elegans and less than in rice) yielded evidence for mechanisms additional to the genetic code, to generate the phenotypic diversity in metazoans. These mechanisms include transcriptional regulation by non-coding RNA, alternate splicing, and post-translational protein modification. In addition, epigenetic mechanisms may well play an important role in gene regulation during growth and development. Epigenetic modifications of the nucleosome depend on the cellular microenvironment. Thus, environmental conditions may affect gene regulation during normal and likely deregulated growth. So far, stem cells have still provided a safe harbour for the genetic paradigm by identification of sentinel transcription factors. However, recent evidence suggests that stemness is just a state and that state and fate of a stem cell are epigenetically regulated and affected by microenvironmental conditions. Moreover the stem cell paradigm has recently gained importance in cancer research.

Epigenetic mechanisms in cancer
Gene expression is regulated by genetic and epigenetic mechanisms, i.e. transcription factors and chromatin modifications. Of interest, chromatin modifications are inherited from mother to daughter cells. Epigenetic mechanisms are now also well known to be associated with cancer (3, 4). For example, accumulation of CpG DNA hypermethylation in the promotor regions of tumor suppressor genes results in loss of their expression in various cancers of adults including lung (5), breast, prostate. Moreover, chromatin modifications, in particular histone alterations via changes in the global pattern of acetylation and methylation of histone H3 and H4 are implicated in tumor development (7, 8). Various protein groups with disparate functions participate in gene regulation by chromatin modifications: Methylation (me) of lysine 4 in histone H3 (H3K4) by members of
the trithorax group (TrxG) under most circumstances increases, whereas meH3K27 by members of the polycomb repressor group (PcG) restrains gene expression. The mixed lineage leukemia (MLL) gene is a prominent representative of TrxG. MLL fusion products lacking the meH3K4 Su(var)3-9, enhancer of zeste, trithorax (SET) domain mediate stemness and malignancy in childhood acute leukemia. Stemness and malignancy are possibly mediated by recruiting the expression activating H3K79 methyltransferase DOT1* au lieu of repressing wild type MLL, leading to aberrant expression of meH3K79 marked HoxA clusters (9).

Gene silencing by PRC2

Herein, much has been learned of gene silencing by histone methylation. The histone methyltransferase Enhancer of Zeste, Drosophila, Homolog 2 (EZH2) is part of the polycomb repressor complex 2 (PRC2) together with Embryonic Ectoderm Development (EED) protein and Suppressor of Zeste (SUZ12). Within PRC2, EZH2 exhibits methyltransferase activity and silences target genes by methylating lysine 27 on histone 3 (H3K27) and lysine 26 on histone H1 (H1K26). EZH2 must be complexed with at least two of its noncatalytic partners, EED and SUZ12, to attain robust histone methyltransferase activity (10, 11). EZH2 is already active at gastrulation and maintains a stemness expression signature. Through genome-wide studies it is now well appreciated (12, 13), that frequent PRC2 targets are transcription factors and signalling components with key roles in cell fate decisions in a wide variety of organisms. PRC2 and H3K27 methylation are also implicated in mammalian X-chromosome inactivation (14).

In tumors, the common finding is that EZH2 is abnormally elevated with most significant increases at advanced stage of the disease and in patients with poor prognosis (15-17). It was also demonstrated that EZH2 may recruit DNA-methyltransferase to promoter regions to permanently switch off genes (18). However, already Varambally (15) observed that EZH2 mediated gene silencing could be reverted by histone deacetylase inhibitors, since PRC2 complexes interact through EED with histone deacetylase 2 (HDAC2) to mediate their suppressive activity. In addition, EZH2, SUZ12 and EED were also detected in the cytoplasm of eukaryotic cells and implicated here in controlling actin polymerization in response to signal transduction (20). In prostate cancer, cytoplasmic overabundance and knockdown suggest that EZH2 influences invasiveness and F-actin polymerization in these cells (21, 22). Thus, both nuclear and cytoplasmic functions may contribute to EZH2-mediated alterations in cancer cells.

Ewing Tumors

Ewing Tumors (ET), alias Peripheral Neuro-Ectodermal Tumors (PNET) or Malignant Peripheral Neuroectodermal Tumors (MPNT), are highly malignant, localized in bone or soft tissue and are molecularly defined by ews/ets translocations. The histogenetic origin of the ET stem cell has been a matter of debate since the original description by Ewing in 1921. Ewing originally described the tumor as diffuse endothelioma of the bone. Recently, we identified a relationship of ET to both endothelial and fetal neural crest-derived cells after having demonstrated neuroectodermal histogenesis of ET in 1985. Self organizing maps and hierarchical clustering revealed an expression pattern of ET of high similarity between ET and fetal, neuronal as well as endothelial tissues. These findings supported the concept that a primitive neural crest-derived progenitor at the transition to mesenchymal and endothelial differentiation is transformed in ET. Moreover, these analyses identified a number of genes found to be over-expressed or even specifically expressed in ET.

Regulation of PRC2 complex in Ewing Tumors

One of those genes strongly induced in ET was the histone methyltransferase EZH2 (24, 26). This analysis initially obtained with custom arrays was verified in an independent analysis on HG U133A arrays (Affymetrix). The latter analysis similarly demonstrated, that the expression of other members of PRC2 complex, specifically EED and SUZ12 was not deregulated in ET. Furthermore, we observed EWS-FLI1 bound to the EZH2 promoter in vivo by chromatin-IP and induced EZH2 expression in ET and mesenchymal stem cells. When we down-
regulated EWS-FLI1 in ET lines by specific siRNA (28) only the expression of EZH2 was suppressed. We cannot exclude that EWS-FLI1 may also bind to the promoters of EED and/or SUZ12. However, abrogation of EZH2 expression by down-regulation of EWS-FLI1 together with the results obtained with the microarray data suggests that only EZH2 of PRC2 complex is deregulated or targeted by EWS-FLI1 in ET.

Expression of EZH2 is essential for tumor growth and metastasis

When we down-regulated EZH2 in ET by specific siRNA these cells lost their ability of contact independent growth in colony forming assays. In addition, cell proliferation of ET lines was blocked by histone deacetylase inhibitor (HDACi) Trichostatin A (TSA), while growth of neuroblastoma and pediatric ALL lines was not inhibited. Based on these results we asked whether modification of EZH2 in ET might also affect tumorigenic growth in vivo. We observed that suppression of EZH2 expression resulted in a significant delay of tumor growth in immunodeficient Rag2⁻/⁻γc⁻/⁻ mice. Similarly, the metastatic behavior ET cells was strongly influenced by the level of EZH2 expression: while control cells injected into the tail vein strongly colonized the lungs and liver, cells with repressed EZH2 almost completely lost their ability to colonize the lung and liver tissue.

EZH2 maintains a stem cell phenotype in Ewing Tumors

ET are composed of highly undifferentiated, small round blue cell tumors containing a vesicular nucleus and a small cytoplasm with a sparse intercellular stroma. When we blocked EZH2 expression in ET lines by RNA interference or treated them with HDAC inhibitors and subsequently investigated their genome wide expression pattern by microarray analysis we observed an undifferentiated, reversible phenotype maintained by EZH2 in ET. Genes typically expressed in neuronal or endothelial cells e.g. epithelial membrane protein 1 (EMP1), Ephrin receptor B2 (EPHB2), glial fibrillary acidic protein (GFAP), growth associated protein 43 (GAP43), protocadherin 7 (PCDH7) or activated leukocyte cell adhesion molecule (ALCAM) were up-regulated by EZH2 suppression, whereas stemness genes such as nerve growth factor receptor (NGFR) were repressed. Furthermore, EZH2 suppression increased the ability of this tumor to develop endothelial or neuronal differentiation potential when analyzed in respective differentiation assays.

These data suggested that EZH2 might play a central role in ET pathology by sustaining the stem cell phenotype of this tumor. This is in contrast to tumor suppressor genes in colon cancer that were shown to be methylated irreversibly in their promotor region even after depletion of EZH2 (29) and indicated to us a more comprehensive role for EZH2 in tumorigenicity by regulating a reversible state of gene expression in ET. Moreover, this finding indicates that not all changes mediated by EZH2-containing PRC2 in tumor cells are necessarily maintained due to promotor DNA methylation (30-32). This supplements previous findings in prostate cancer (33). However, why such a reversible state is maintained in ET and whether this involves H3K27 demethylases is not yet known.

Stemness in Ewing Tumors is maintained by epigenetic regulation

A number of stemness genes including NGFR were found to be down-regulated following EZH2 blockade in ET. Whether this involves PRC2 complex activity is not known. In mouse embryonic stem (ES) cells, a role for PRC2 in activating certain target genes has been considered (35), and PRC2 subunits seem to be required in human fibroblasts for the expression rather than silencing of genes important for proliferation (36). Similarly, non-coding RNA may be guiding chromatin-modifying complexes to epigenetically target genes (37, 38). To test the relevance of this approach we suppressed Argonaute-1 (AGO1) that is necessary for this process (39). We again observed a suppression of NGFR expression by about 50% suggesting that non-coding RNA may be involved in the PRC2 mediated induction of target gene expression (26).

Subsequent results suggested that the preservation of stemness is directly mediated by epigenetic mechanisms: Transient or constitutive down-regulation of EZH2 decreased the overall histone H3K27-trimethylation and
increased histone H3-acetylation in ET as observed by western blotting. In addition, transient down-regulation of EZH2 even resulted in gene specific reduction of H3K27-trimethylation as observed by ChIP-chip analysis and shown for differentiation genes EMP1, EPHB2, and GAP43. Overall, histone H3 density and H3K27me3 coexisted at several loci of the target genes indicating that a repressed chromatin configuration was present in ET. Since this repressed chromatin configuration depended on EZH2 it is possible that EZH2 also bound within the promoter region, an observation that warrants further investigation.

So far, it is not known how EZH2 is recruited to target genes. In *Drosophila*, Polycomb response elements (PREs) have been localized using reporter assays and several DNA-binding proteins are implicated in recruiting PRC2. However, no mammalian PREs to date are identified. A presumed candidate for a mammalian PRC2-targeting factor is YY1, since YY1 is needed for H3K27 methylation of target genes in muscle cells (41). Still, this role may be cell-type specific since there is little overlap between YY1 and PRC2 targets in mouse ES cells (42). Other possible candidates are not yet excluded and more work has to be done to define sequence elements and mechanisms that recruit PRC2 to target loci in mammalian cells.

Components of PRC2 complex have also been detected in the cytoplasm of mouse and human cells and methyltransferase activity has been implicated in the control of actin polymerization in response to cell signaling (20). Moreover, cytoplasmic overabundance of EZH2 has been identified in prostate cancer. Therefore, increased EZH2 expression in ET may affect cytoskeletal-based features including migration and invasiveness of tumor cells congruent with our observation of strongly reduced lung metastases after EZH2 knock down.

**Clinical implications**

Our findings further indicate that EZH2 and EZH2-mediated events are promising novel pharmacological targets for ET therapy and potential inhibitors of EZH2 histone methyltransferase activity are emerging. One reported drug is deazaneplanocin A (DZNep) that acts by an indirect mechanism (43). In breast cancer cell lines DZNep can deplete PRC2 subunits and reactivate PRC2-silenced genes. Similarly, physical and functional links between EZH2 and histone deacetylases (HDACs) (19, 44) are well established. In human cells, PRC2 can physically associate with HDACs 1 and 2 (19, 45) and EZH2 knock down or Trichostatin A treatment in ET results in the induction of a similar set of genes. Confirmation of these results with HDAC class 1 inhibitor (HDACi) MS-275 alluded to a similar effectiveness in blocking the stemness phenotype of this tumor indicating that epigenetic therapy with HDACi could revert or arrest processes contributing to the pathology of this tumor. However, since these type of inhibitors could affect many processes that require either methyl transfer or acetylase activity, it raises concerns about unspecific side effects that need to be addressed in future preclinical and clinical studies.

**Biomedical Implications:**

**A contextual concept of stemness and malignancy in embryonal tumors**

Our observations suggest that EZH2 expression is required in Ewing tumors to maintain stemness as well as metastatic behavior. Thus, we have shown how an oncofusion protein utilizes a key enzyme of stemness to serve the needs of the malignant embryonal tumor stem cell. To our knowledge, the interaction of a chimeric transcription factor with EZH2 represents a novel mechanism of epigenetics in cancer. In ET, methylation of target genes and histone modifications are apparently driven by a translocation, originating from fetal or embryonic tissue rather than by exogenous events yielding dedifferentiation of differentiated cells (Figure 1). Moreover, involvement of EZH2 in neuroectodermal and endothelial malignancy has not been described previously as well as contribution to metastasis appears to be another novel function for EZH2.
A chromosomal translocation product maintains stemness in Ewing Tumors by induction of an epigenetic key protein. ET-specific ews/fli translocation results in an aberrant EWS-FLI1 fusion protein able to bind to the promoter region of ezh2, thereby directly influencing epigenetic activity of PRC2 complex. This activity results in the repression of endothelial and neuroectodermal differentiation and preserves a stemness phenotype in ET (Stephanie Plehm modified from).

Taken together our results link genetic and epigenetic mechanisms in the cancer stem cell concept of this embryonal tumor. The genetic paradigm is no longer sufficient to explain stemness in cancer stem cells, in particular in embryonic cancer stem cells. A single genetic alterations rather affects global stemness programs by altering crucial epigenetic pathways. These findings provide a novel glimpse at the importance of epigenetic mechanisms in transformation by somatic gene mutations, in particular chromosomal translocations.

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27. A complete reference list of all cited articles can be found in the original document.


Rationale for Tumor Prevention of HPV Vaccination in Adolescents

Luisa Lina Villa

Abstract

Human papillomavirus (HPV) is responsible for all cases of cervical cancer, as well as a great percentage of other anogenital tumors and oropharyngeal tumors. Since the main etiologic factor for these diseases is a virus, prophylactic measures are the best way to reduce the burden caused by the infection and associated disease. Since HPV infections are acquired very early at the onset of sexual activity, vaccinating children and adolescents previous to sexual debut is considered to be the most effective way to prevent infection ad disease caused by common HPVs. This review brings up to date information on the two commercially available prophylactic HPV vaccines against HPV, as well as discussing future directions and challenges for HPV education and adolescent immunization in both developed and developing countries.

New prophylactic HPV vaccines have the power to prevent many HPV infections, thus reducing the burden of HPV-associated diseases. Two vaccines have been developed, a quadrivalent vaccine that protects against HPV 16, 18, 6, and 11, (GARDASIL, Merck Sharp and Dohme), and a bivalent vaccine that protects against HPV 16 and 18 (CERVARIX, Glaxo SmithKline). Both vaccines are composed of HPV L1 proteins that have spontaneously self assembled into virus-like particles (VLPs). However, they have different manufacturers, valencies, adjuvants and are produced in different types of cells. Both are administered by intramuscular injection, in three doses (0, 1 or 2 and 6 months). The types and levels of immune responses generated by both vaccines may vary. Importantly, however, both vaccines have shown very high efficacy to prevent infection and disease caused by the types included in the vaccine. Several clinical trials of these prophylactic HPV vaccines have being conducted in different countries including about 50,000 individuals. All of the trials were blinded, randomized, and placebo controlled trials of young women (mean age 20). Other trials are still ongoing that include adult women and men. Prophylactic efficacy was measured considering HPV infection and disease endpoints, particularly Cervical Intraepithelial Neoplasia grades 2 or worse (CIN2+) for the bivalent and quadrivalent vaccines, as well as Vulvar Intraepithelial Neoplasia (VIN) or Vaginal Intraepithelial Neoplasia (VaIN) and genital warts, for the quadrivalent vaccine only. The per-protocol populations included women who were naïve at baseline to HPV 16 and 18, or to HPV 6, 11, 16, and 18, as determined by serology testing for presence of HPV type-specific antibodies or polymerase chain reaction (PCR) testing of genital samples for the presence of HPV DNA. For both the bivalent and quadrivalent vaccines, results of different trials allow for the examination of broad trends in efficacy in preventing HPV 6/11/16/18-related disease in several groups of patients categorized according to their HPV status at baseline. The quadrivalent vaccine was 100% effective in reducing the incidence of HPV 6/11/16/18-related disease in several groups of patients categorized according to their HPV status at baseline. The quadrivalent vaccine was 100% effective in reducing the incidence of HPV 6/11/16/18-related disease in several groups of patients categorized according to their HPV status at baseline. However, there was no clear evidence of protection from disease caused by HPV types for subjects that were HPV DNA positive by PCR. Similar results were obtained for the bivalent vaccine. In fact, vaccination of HPV16/18 DNA positive women does not enhance clearance of the viral infection. In a recent publication of a phase III trial, this bivalent vaccine showed 90% prophylactic efficacy against CIN2+ associated with HPV 16 or HPV 18. In a combined according
to protocol analysis of women with evidence of current or past infection with one or more of the vaccine targeted HPV types, the quadrivalent vaccine was 100% effective at preventing CIN2+ or AIS associated with vaccine targeted types to which the vaccine had no evidence of prior exposure. Thus prior or prevalent infection by one type does not appear to influence the effectiveness of the vaccine against other types.

Results on protection against infection by non-vaccine HPV types have been published and reviewed in Ault (2008). Cross-protection against incident infection with HPV types 45 and 31 has been observed for both the bivalent vaccine and the quadrivalent though at different levels. Further studies to better understand the significance and durability of these responses are warranted. It is possible that the best and most efficient responses will be obtained with multivalent vaccines against a larger number of genital HPV infections which are presently under development.

VLPs are noninfectious protein subunit vaccines and therefore might be expected to have safety profiles similar to other protein subunit vaccines such as tetanus or hepatitis B vaccines. Both vaccines were generally well tolerated and there were very few dropouts due to vaccine-related symptoms. The most common vaccine related adverse events were local transient mild to moderate pain and erythema at the site of injection. The proportion of women experiencing serious adverse events, whether deemed to be related to the vaccine or not, was much the same in VLP vaccines and controls. Although the women were encouraged to use reliable methods of birth control, pregnancies did occur in numerous women enrolled in the trials. Overall, there was no difference in pregnancy outcomes, such as proportions of live births, spontaneous abortions or congenital abnormalities, between VLP vaccines and controls for either vaccine.

Since the VLP vaccines were designed primarily to protect by inducing virion neutralizing antibodies, type specific antibody responses to the VLPs have been the primary focus of immunogenicity studies. Both vaccines were shown to be highly immunogenic in the clinical trials, resulting in essentially 100% seroconversion in the different populations studied. Peak geometric mean antibody titers (GMTs) were approximately 10- to 100-fold higher that the GMTs generated after natural infection. Both vaccines induce the expected B cell memory response which is a property of vaccines with durable immune responses. Although the long term persistence of stable antibody levels is an encouraging finding, the antibody levels needed to prevent infection or disease are currently unknown. However, long-term follow-up studies have shown that efficacy is maintained for at least 7 years, and modeling studies suggest protection may last much longer. The available data do suggest that HPV vaccines would provide a lengthy period of protection, likely to usher a vaccinated individual through the years of highest infection risk and beyond.

Because HPV infection is most common among young, sexually active individuals, the vaccine will be most effective if administered to individuals previous to onset of sexual activity. It was therefore critical to demonstrate the safety and immunogenicity of the HPV vaccines in younger individuals than those women in whom the vaccine efficacy has been demonstrated. In two immunogenicity bridging studies, the quadrivalent vaccine was shown to be safe and immunogenic in adolescent boys and girls (9-15 years old). The antibody response to the vaccine was approximately two-fold higher in this age group than the responses in young women. Similar profiles of safety and immunogenicity in adolescent girls and young women were described for the bivalent vaccine.

In these adolescent and adult studies, vaccination has been well-tolerated. Extensive safety information has been published. This includes data on pregnancy outcomes, as well as all serious adverse events, and systemic adverse events, categorized by organ system. Constant updates on the safety of the quadrivalent HPV vaccine can be found at the American Center for Diseases Control (CDC). Substantially more information on the performance of the vaccine in males, older women and immunosuppressed populations should become available in the next few years as clinical trials in these groups are completed.

Based on its demonstrated clinical efficacy and favorable safety profile, HPV prophylactic vaccines are being introduced in many countries...
around the World, including developed and developing countries. The rapid approval and launch of such vaccines are a clear indication that governments and policy makers are aware of the expected impact on the prevention of one of the most common causes of female mortality worldwide. However, incorporation of HPV vaccination in the public health sector is still to be seen in the developing World, mostly due to vaccine cost. HPV vaccine implementation will also depend on local infrastructure for vaccine delivery to the initial target population during the window of highest vaccine efficacy, i.e. prior to sexual exposure. Furthermore, introducing HPV vaccines in the present cervical cancer control system is hampered by the fact that secondary screening with Pap tests (or HPV DNA testing) will still be required to detect cervical cancers and pre-cancers caused by non-vaccine HPV types. Ongoing cost-benefit studies and negotiations between governments, the private sector, and non-governmental organizations may enable some of the developing countries, where the vaccine is most needed, to implement the necessary programs. In addition, vaccinated populations should be followed-up for long-term safety, sustained immune responses and vaccine disease efficacy. Effective monitoring will benefit from linkage of vaccination history and screening history, as well as precise measurement of HPV infection, both DNA and serological testing.

Education of physicians, policy makers, parents, and adolescents will be crucial for delivering HPV vaccines which ultimately will result in the reduction of cervical cancer rates and other HPV-related diseases worldwide. Vaccine acceptance is largely determined by health beliefs, such as the individual’s perceived susceptibility to the disease; vaccine characteristics, such as cost and efficacy; and obstacles to obtaining the vaccine. Health perceptions are expected to play a major role in the acceptance of HPV vaccine, as is the fact that the vaccine raises the morally and politically charged issue of adolescent sexual behavior. Physician attitudes are extremely influential to both parents and adolescents, and perception that the physician regards the HPV vaccine as important and recommended will be a critical step towards vaccine acceptance. Altogether, education of physicians, parents, and adolescents will be crucial for delivering HPV vaccines to target populations during the window of highest vaccine efficacy, prior to sexual debut.

**Summary**

Two commercially available vaccines have been shown to be safe, highly immunogenic and efficacious in preventing diseases related to most common HPV types. HPV infection is most common among young, sexually active individuals. In clinical trials, both vaccines were shown to be safe and immunogenic in adolescent boys and girls (9-15 years old), with immune responses even higher than those observed for young adults. Education of physicians, parents, and adolescents will be crucial for delivering HPV vaccines to target populations during the window of highest vaccine efficacy, prior to sexual debut.

**References**


Oncogenic Pathways in Embryonal Tumors

Angelika Eggert

Abstract
The complexity of cancer phenotypes is characterized by multiple mutations and alterations in the cancer genome. A consequence of these alterations is the deregulation of various signaling pathways controlling cell function. Molecular profiling studies have the potential to describe this complexity, and provide an opportunity to link pathway deregulation with potential therapeutic strategies. This lecture will provide an overview of current genomic and proteomic data, which have expanded our knowledge of oncogenic pathways, their functional roles and interactions in embryonal tumors. The suitability of targets identified within these oncogenic pathways for drug development will be discussed.

Introduction
The progression of pediatric cancer therapy to the current overall high cure rates has been a milestone in the history of modern oncology, and has impacted treatment design for adult cancer patients. Approximately 30% of childhood malignancies are embryonal tumors, including neuroblastomas, medulloblastomas, Ewing sarcomas, retinoblastomas nephroblastomas and hepatoblastomas. The group of embryonal tumors has progressed in the past 40 years from being virtually treatment-resistant to having an overall 5-year survival of 70% of patients, mainly due to improvements of cure rates in early tumor stages. Nevertheless, treatment of disseminated disease frequently fails, and results in survival rates < 20%. Thus, treatment of embryonal tumors remains a challenge for the pediatric oncologist.

The traditional approach to improving tumor therapy is adjusting the treatment intensity for the individual risk of relapse to maximize survival and minimize long-term morbidity. Risk stratifications based on tumor histology and cytogenetics have improved therapy success. However, the prognostic predictive value of currently available diagnostics is insufficient. The presently defined risk groups, even within the same tumor entity, are associated with heterogeneous treatment outcome. Identifying novel genetic or proteomic signatures can provide pediatric oncologists with more meaningful diagnostic tools, and possibly to allow new subclassifications of the different embryonal tumor entities.

Single molecular technology platforms including microarrays and arrayCGH have been validated in comparison to classical cytogenetics in recent, more conventional approaches. Current research strategies go beyond these approaches by combining clinical, cytogenetic and molecular characterization with several of the most advanced technologies in expression profiling and proteomics to provide additional diagnostic panels. Such an approach requires the transnational integration of available resources and technologies.

Research focused on embryonal tumors in the European consortium “E.E.T.-Pipeline”

The European research consortium, the “E.E.T.-Pipeline”, provides a comprehensive multi-team approach to improve embryonal tumor diagnostics and treatment by the integration, assessment and validation of information generated by basic research utilizing high-throughput technologies. This integrated, post-genomic research effort channel these effort via dual pipelines concentrating on (1) state-of-the-art diagnostics and (2) innovative drug development and preclinical testing. A further gain of knowledge is achieved by meta-analysis integrating newly obtained and existing data derived from different genomic and proteomic platforms for the embryonal tumor entities.
This is a novel approach, as most conventional analyses have regarded each embryonal tumor as a separate entity, a strategy not completely supported by the biology of this tumor group. Morphologically related tumor entities sharing similar emergence and progression patterns are likely to share common molecular pathways as well as druggable targets for therapeutic intervention. Some of these common oncogenic pathways and targets have already been identified for embryonal tumors. Defining the common elements of tumorigenesis among embryonal tumor entities, rather than what divides them, will provide the best opportunity for common diagnostic tool and drug development in pediatric oncology.

Embryonal tumors provide unique features of tumor biology including a high incidence of spontaneous regression and differentiation as well as features of common interest in cancer biology including genetic changes leading to therapy resistance, enhanced tumor angiogenesis, proliferation, migration, invasion and metastasis. The early manifestation of embryonal tumors in infants and young children suggests that only a limited number of genetic changes lead to the transformed phenotype, making these tumors an ideal model for the post-genomic investigation of cancer-related expression changes.

Previously published cDNA and arrayCGH-based studies have given insight into potential therapeutic targets, although as yet, this information has not been translated into the development of novel drugs. This is mainly due to the fact that beyond defining characteristic gene expression signatures, only a limited number of studies have addressed the functional analysis of identified target genes. The lack of validated post-genomic technology available for routine diagnostics and a considerable gap between target identification in basic research efforts and the resulting preclinical development of urgently needed novel drugs are the limitations of current treatment approaches.

It is likely that distinct disease-specific initiating mechanisms exist for different malignant diseases, but that these converge on common downstream pathways necessary for propagation and spread of the cancer. The identification of shared pathways within a group of similar rare diseases such as the embryonal tumors may provide targets for drug development that are more economically feasible for pharmaceutical companies. The resulting drugs would be more likely to go into clinical trials because of the increased market and the higher likelihood that critical numbers of patients could be entered into clinical trials in a reasonable timeframe.

Oncogenic pathways in embryonal tumors: c-myc, MYCN, EWS-FLI1, RB and more

Embryonal tumors are excellent examples to demonstrate and analyze the importance of oncogenes for tumor development and progression. Amplification or activation of the tissue-specific MYC family genes, c-myc and MYCN, as well as alterations in downstream cell cycle regulatory genes contribute to the genesis of a wide variety of human tumors including neuroblastoma, medulloblastoma and Ewing sarcoma, and are major oncogenic pathways in these entities. The MYC proto-oncogenes encode nuclear phosphoproteins involved in the transcription of genes central to regulating cell cycle, proliferation, differentiation and apoptosis. MYC expression is normally tightly regulated throughout the cell cycle, but may become deregulated or activated contributing to malignant transformation. Activated MYC expression due to either gene amplification or transcriptional induction causes aberrant expression of several cell cycle control genes including cyclins D and E, eventually resulting in hyperphosphorylation of the retinoblastoma protein, pRb, and entry into DNA replication. Previous studies have identified c-MYC, N-MYC, and pRb pathway components as being commonly altered in embryonal tumors by entity-specific mechanisms. These changes are also frequently associated with poor prognosis.

Ewing’s Sarcoma

The genetic lesions of the cell cycle machinery converging on MYC activation and pRb phosphorylation differ between individual embryonal tumor entities. In Ewing’s sarcoma, the primary insult is the EWS-FLI1 gene rearrangement, which has been most intensively investigated functionally. There is ample experimental evidence that Ewing’s sarcoma growth and tumor cell survival is absolutely
dependent on the sustained functioning of EWS-FLI. Comparing several different Ewing’s sarcoma cell lines, the c-MYC pathway and replication licensing, which initiates the G1 phase of the cell cycle, have been consistently found to be deregulated by EWS-FLI1. Preliminary evidence suggests that ectopic c-MYC expression can rescue Ewing’s sarcoma cells, in which EWS-FLI1 expression and consequently endogenous c-MYC expression is silenced by an RNA interference (RNAi) approach. Thus, the c-MYC pathway is also an attractive target for drug development to treat Ewing’s sarcoma.

**Medulloblastoma**

Similarly, in medulloblastoma, the most common malignant childhood brain tumor, retrospective evidence was provided that c-MYC might be a prognostically and therapeutically important target. Because of the high risk of leptomeningeal dissemination, standard postoperative treatment for medulloblastoma includes not only local radiotherapy, but also craniospinal radiotherapy and chemotherapy. Such treatment causes long-term morbidity including endocrine and growth disturbances, as well as neurocognitive dysfunction, which is particularly severe in young children. Markers distinguishing children that may profit from less aggressive from those requiring intensive treatment may help to reduce morbidity in survivors. According to retrospective studies, high TrkC neurotrophin receptor mRNA expression and low c-MYC expression may provide powerful independent predictors of a favorable survival outcome in medulloblastoma patients. Up-regulation of c-MYC in poor prognosis MB, which is independent from gene amplification in >95% of the cases, may involve the Wingless/Wnt pathway, as has also been suggested for Ewing sarcoma. Other mechanisms of c-MYC activation in medulloblastoma are mutations in either APC or â-catenin, which lead to stabilization of the â-catenin protein, its nuclear accumulation and subsequent induction of c-MYC. In a recent study, aberrant nuclear â- catenin staining has been demonstrated in 18% of sporadic medulloblastoma.

**Neuroblastoma**

In neuroblastoma, the amplified MYCN oncogene is a strong prognostic marker for poor outcome, and is the primary cause of aberrant cell cycle regulation. However, a substantial number of aggressive neuroblastomas lack amplified MYCN and prognosis prediction in this tumor subset at the time of diagnosis is often difficult. By analyzing the downstream pathway of MYCN, a subset of MYCN and E2F target genes has been identified that are aberrantly expressed not only in MYCN-amplified tumors but also in high-risk MYCN single-copy tumors even in the absence of deregulated N-MYC mRNA or protein expression. A disrupted Rb-Skp2 pathway was identified as a common mechanism leading to aberrant MYCN and E2F target gene levels, and results in aberrant activity of Skp2, a member of the SCF<sup>Skp2</sup> ubiquitin ligase family. Deregulated Skp2 has a proteolytic-dependent function involved in the degradation of p21 and p27, as well as a proteolytic-independent function as a transcriptional activator of N-MYC and probably also E2F target genes. These results imply that combined targeting of Skp2 and N-MYC functions in neuroblastoma could be a promising new therapeutic approach for this tumor.

**Summary**

Taken together, the distinct mechanisms of activation of common cell cycle regulatory pathways have been investigated in individual embryonal tumor entities within the framework of the European research consortium, the “E.E.T.-Pipeline”. Pathway components that best serve as reliable prognostic markers in these diseases as well as common pathway components that may be best targeted by therapeutic agents have been identified. Functional read-out systems for drugs targeting these pathway components have been established to further improve preclinical drug development for embryonal tumors.

**References**


