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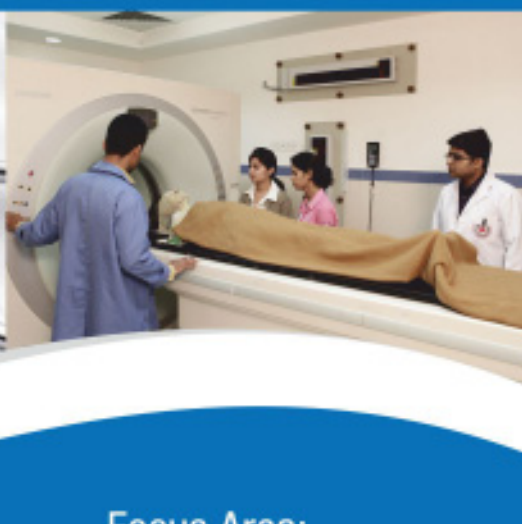

Focus Area:

CHILDHOOD CANCERS

Rajiv Gandhi Cancer Institute and Research Centre

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From the Desk of Director Research

Progress in Pediatric Oncology has been one of the biggest success stories in oncology in the last millennium. The 5-year survival for all pediatric cancers is now 75- 80%. Childhood cancer constitutes less than 5% of the total cancer burden in India, with approximately 45,000 children being diagnosed with the disease every year. In India, 1.6-4.8% of all cancer cases occur in children less than 15 years of age with variation by place of residence. The majority of children with cancer require rounds of chemotherapy to treat their disease. Many require surgery and/or radiation to remove the tumor. For some, a bone marrow transplant is the only hope of survival. As more and more children are surviving cancer, it is increasingly important to address ongoing and emerging needs of children and their families in order to achieve complete and long term healing.

The outcome of pediatric cancer has gradually improved in the country over the last four decades. The outcome of hematological cancers in terms of long-term survival has greatly improved from 20% to 60% in ALL, from <70% to >90% in Hodgkin's disease, from 30% to 70% in non Hodgkin's lymphoma and from 10% to 40% in acute myeloblastic leukemia. Similarly, the outcome in solid tumors has also improved. Further research is required to develop less toxic treatments for the curable pediatric cancers and newer approach for patients currently having dismal outcomes.

Cancer care thus remains a challenge. In India, there is an urgent need to appreciate the fact that cancer in children is highly curable in expert hands .The fundamental steps in caring for these children are to estimate the current burden, understand and overcome the barriers, and develop strategies to detect early, refer and treat cancers with appropriate expertise. Public understanding about the prevalence and treatability of childhood cancers in the developing world can enjoin governments to take action and on drug companies to moderate drug costs. In an ideal world, children with cancer should have access to the best treatment and pain control, and be enabled to go to school and take part in normal life as far as possible. We must hope that feasible initiatives by the concerned to meet the need of the vulnerable children with a life-threatening disease would contribute to an improved environment for all children.

The present issue of the Cancer News highlights the newer advances in the field of "Pediatric Cancer" and features regular articles, such as Special Feature, Guest Article, Emerging Scenario, Perspective, Recent Advances and In Focus. We are grateful to Dr Jayant Maini and Dr Vani Brahmachari of Dr B R Ambedkar Center for Biomedical Research, University of Delhi and Dr Mammen Chandy and Dr Shekhar Krishnan of Blood and Marrow Transplant Division, Tata Medical Centre, Kolkata, for providing us articles.

Suggestions/ comments from the readers are welcome.

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SPECIAL FEATURE

DECIPHERING THE HUMAN GENOMIC LANDSCAPE OF CHILDHOOD CANCERS

In recent decades pediatric oncologists all over the world have realized the importance of somatic genomic alterations as an important component of diagnosis, prognosis, and therapy selection in childhood cancers. Therefore, their focus of research involves identification of the full spectrum of driving mutations causing pediatric cancers and its incorporation into clinical practice so as to provide personalized treatment to every child. Conventional tests using karyotyping, fluorescent in situ hybridisation (FISH) and polymerase chain reaction (PCR) to identify known genomic alterations and their application in risk stratification and treatment is standard of care for most of the pediatric cancers. Recent technological advances like next generation sequences (NGS) have provided overwhelming genomic information pertaining to childhood cancers. This information has revealed an unprecedented view of the tumor genome, thus enabling clinicians to have a better understanding of the pathobiology of this disease. The biggest challenge is to select the clinically significant genomic alterations out of this mass of information. This huge data may enable researchers to discover drugs targeting disease specific molecular changes and many trials are underway to test novel agents. Hence, we may hope that in the near future molecularly targeted therapies may valuably add to the arsenal available for treating childhood cancers, both in terms of improving survival and reducing treatment related toxicities.

Spectrum of Molecular Profiling in Childhood Cancers

The spectrum of childhood cancers is entirely different from that of adult malignancies. It differs not only in the type of cancers that predominantly occur during childhood but also in the biology of disease. Therefore, it is not surprising that the spectrum of mutations leading to malignant transformation also differs between pediatric and adult cancers. Unlike adults, in most pediatric tumor types, only 5% to 15% of cases have point mutations, translocations, or copy number alterations in genes constituting viable therapeutic targets. In addition, pediatric cancers have more frequent amino acid changing point mutations per case, such as neuroblastoma, medulloblastoma, ETP-ALL, and osteosarcoma; point mutations tend to occur across a number of genes and few genes are recurrently mutated in a high proportion of cases. Moreover, at least in some pediatric cancers, alterations in the epigenome, rather than accumulation of point mutations in coding genes, may be the primary mechanism of oncogenesis.

Advanced Molecular Testing in Pediatric Cancers

Somatic genetic analysis using common tests like karyotype, FISH, and polymerase chain reaction have refined risk stratification, prognosis and treatment of certain subsets of pediatric cancers. MYCN amplification in neuroblastoma, bcr-abl in ALL and PML-RARA in acute promyelocytic leukemia are prime examples of this. However, owing to the low frequency and high variability of oncogenic targetable genomic events benefits of these are limited to very few patients. Newer techniques like gene sequencing may overcome these shortcomings by deep sequencing of many genes simultaneously in a relatively short time and at an acceptable cost. Pediatric Cancer Genome Project (PCGP), co-led by St. Jude Children's Research Hospital and the Washington University School of Medicine aims to discover the genetic origins of select pediatric cancers and has recently completed whole genome sequencing of 600 childhood cancer cases.

Application of Newer Molecular Techniques

Newer molecular technique platforms perform massively parallel sequencing, during which millions of fragments of DNA from a single sample are sequenced in unison. Massively parallel sequencing technology facilitates high-throughput sequencing, which allows an entire genome to be sequenced in less than one day. This information may be used for functional and therapeutic genomics.

Functional genomics: By NGS we get enormous data with regard to genomic alterations of which only some are thought to initiate and contribute to the genesis of an oncological event. So one has to identify these "driver" mutations from innocent bystander "passenger" mutations, which are random background mutations that are not relevant to the disease. Identification of these driver mutations will help to design customized panels

containing selected genes of interest for specific diseases. So far NGS has enabled detection of several novel and rare somatic relevant mutations associated with childhood cancers as discussed below.

Acute leukemia: Risk-adapted therapy has contributed to dramatic improvements in childhood acute lymphoblastic leukemia (ALL). Identification of novel adverse factors by gene sequencing is paving the way to more accurate risk stratification by incorporation of these findings. Two such important adverse genetic alterations identified in B-ALL are IKZF1 deletion resulting in the BCR-ABL–like subset and overexpression of CRLF2. In T- ALL, recognition of complex structural variations, focal deletions and sequence mutations of genes encoding key hematopoietic regulators has enabled investigators to categorise an aggressive subtype of pediatric ALL known as early T-cell precursor leukemia.

In acute myeloid leukemia (AML) as well, identification of FLT3 internal tandem repeats and MLL-rearrangement with mutated IDH1/2 are being recognized as high-risk subtypes in contemporary clinical trials.

Pediatric solid tumor: In general, with multimodality approach, the outcome of most pediatric solid tumors, has improved substantially in the last two decades. However, for most metastatic and recurrent solid tumors the results remain unacceptably poor. NGS may well provide us with unique genetic information specific for this subset. The various genomic alterations identified in the common pediatric solid tumors are listed in Table 1.

Therapeutic genomics: The outcome of children has improved tremendously in the past two decades as a result of coordinated systematic co-operative trials. Therefore, it is sometimes difficult to justify inclusion of novel targeted therapy in first line treatment. However, it is definitely the need of the hour for high-risk patients. Additionally, it can be used in good risk patients to reduce the doses of cyotoxic chemotherapeutic agents. The goal of genomic research is to identify clinically relevant genetic alterations which may be targets for therapeutic intervention to improve the survival of high-risk patients and reduce the intensity of treatment for good risk patients. Few molecules have already been developed to target these genetic alterations identified by NGS and are being tested in various clinical trials across the world. Currently, commonly used targeted drugs include: kinase inhibitors, mTOR inhibitors, smoothened/SHH pathway inhibitors, and NOTCH inhibitor. Some of the ongoing trials are mentioned below.

Children's Oncology Group is conducting a phase I/II study of crizotinib, an ALK/c-MET inhibitor, in anaplastic large-cell lymphoma and inflammatory myofibroblastic tumor and neuroblastoma all of which have ALK rearrangements.

Other trials evaluating targeted therapies in molecular subgroups of pediatric cancers include a phase I/II trial

Table 1: Genomic Alterations in Common Pediatric Solid Tumors

	Genomic alteration	**Incidence**
Neuroblastoma	Constitutive activation of the ALK oncogene PTPN1 1	5-10% 1%
Rhabdomyosarcoma	BRAF RAS pathway activation ALK FGFR4	1% 10-40% 50-80% 4%
Osteosarcoma	PIK3CA MDM2	3% 10%
Wilms Tumor	CTNNB1 WTX	15% 20%
Glioblastoma	PDGFRA EGFR	5% 8%
Diffuse intrinsic pontine glioma	PI3KCA PDGFRA	15% 36%
Low-grade glioma	BRAF	50-100%

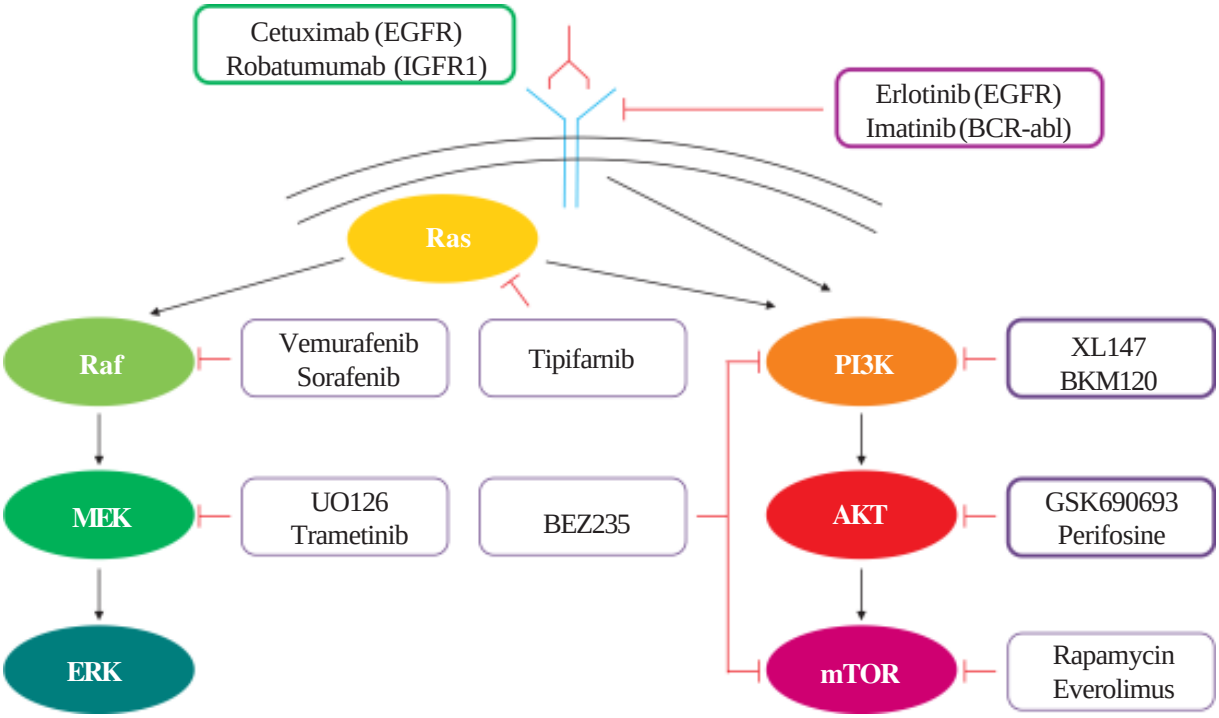

Figure 1: Schematic representation of two main pathways aberrantly activated in pediatric tumors and corresponding targeted therapies (adapted from Saletta et al, BBA Clinical 1; 2014)

of LDE225 in the SHH subgroup of medulloblastoma and a phase II trial of PKC412 in acute leukemias with FLT3 alterations. A randomized phase III study of a new agent, ch14.18, an anti-GD2 monoclonal antibody, in high-risk neuroblastoma has demonstrated improved survival in the group receiving the antibody. Within medulloblastoma, the WNT subtype identifies a patient population with a particularly good prognosis, and trials evaluating therapy reduction are being considered. Erlotinib and gefitinib have been successfully employed to treat high grade gliomas while IGFR mAbs show promising results in pediatric sarcomas. These encouraging results indicate that molecularly targeted therapies have great further potential for the treatment of childhood cancers.

Challenges: A large number of biomarker-matched drugs are tested in clinical trials, and then dismissed. In general, 90% of novel targeted therapies do not transition to routine use, despite good results in the preclinical setting. This can be due to unexpectedly high toxicities, lack of objective efficacy, and/or because agents present no measurable advantages over those already in use. Therefore, we need to identify and target clinically relevant genomic alterations. Moreover, these genetic alterations are not constant and few genes are recurrently mutated in a high proportion of cases. The issues of low frequency and high variability of oncogenic, targetable genomic events make it difficult for therapeutic intervention. In this context, it is important to consider the combination of multiple agents and/or the use of less selective drugs, in order to inhibit multiple pathways at the same time or the same pathways at multiple levels.

Conclusion

Establishing cancer genome sequencing efforts have produced important new insights into the pathobiology of cancer. The rapid generation of genomic data across the landscapes of pediatric cancers is opening new avenues of collaboration. Understanding the functional and clinical relevance of the identified mutations in cancer will require bringing together dedicated teams of genomic and computational experts, oncologists, pathologists, molecular biologists, and others. Though many challenges remain, promising initial results provide hope that the future will bring personalized, less toxic, curative treatments to all children with cancer.

Suggested Reading

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(Dr Sandeep Jain, Consultant, Dr Gauri Kapoor, Director, Dept of Pediatric Hematology and Oncology)

GUEST ARTICLE

PAEDIATRIC HAEMATOPOIETIC TRANSPLANTATION : LOOKING AT THE FUTURE

Blood and marrow transplantation (BMT) represents definitive treatment for a variety of paediatric blood, immune and metabolic disorders. BMT thus serves as substitution therapy, immunotherapy and anti- tumor therapy (Table 1). A successful treatment outcome requires achieving the trifecta of timely haematological and immune reconstitution, enduring correction of the disease phenotype and minimal short- and long-term morbidity. A major hurdle that stands in the way of these objectives is the host HLA (human leucocyte antigen) barrier and the attendant risk of adverse transplant-associated immune events, including graft failure and graft-versus-host-disease (GVHD). The urgency for transplant intervention has thus spurred innovations and refinements in the clinical practice of paediatric BMT (pBMT) to surmount the HLA barrier while limiting the risk of transplant-associated complications. The decision to opt for pBMT treatment however remains patient- specific and requires synthesis of patient, disease, donor and resource variables (Table 2). This decision-making is necessarily a dynamic process and is continually informed by advances in pBMT practice. In this monograph, we briefly discuss these advances, loosely categorising them as recent, evolving and novel for ease of discussion.

Recent Advances

In the past decade, advances in three key areas have improved outcomes in pBMT (Table 3). Preparative regimens have been modified to reduce acute and long-term toxicities. The use of unrelated donors has expanded access to pBMT treatment. Improvements in supportive care, particularly in infection prophylaxis, have enabled safe delivery of these toxic treatments to patients.

Reduced Toxicity of Preparative Regimens

For the most part, preparative regimens in pBMT are myeloablative(1). These regimens are designed to provide cytoreduction, eliminate native haematopoiesis and induce

Table 1 : Outcome Objective in Paediatric Blood an Marrow Transplants

Subsitutation and correction of disease phenotype
acquired and constitutional disorders of haematopoietic elements
Consistutional metabolic disorders
Deficient / aberrant immune function
Sustained Graft-versus-Disease effect
Cytoreduction of preparative regimen
Graft-derived immune clearance / modulation
Timely engraftment and immune recovery
Immune reconstitution (including immune 'reboot')
Sustained Graft-versus-Disease effect
Minimal short and long-term toxicities
Non long-term morbidity
Satisfactory functional status (educational, professional, personal)

Table 2: Decision variablesin paediatric blood and marrow transplants

Factors	Elements	Decision impact
Recipient	Disease status	Fitness for transplant
	Organ function	Choice ofpreparative regimen
	Risk of graft failure	Infection management strategy
	Infection risk	Graft-versus-Disease potenitiation
Donor	HLA-matching	Donor availability
	Graft source & target cell dose	GVHD prophylaxis
	CMV status	CMV-directed strategy
resources	Financial	Overall transplant strategy
	Social (family motivation, support)	(e.g.transplant vs no transplant)
	Medical (expertise, infrastructure)	(matched family vs alternative donor)

CMV : Cytomegalovirus; GVHD : Graft-host disease

Table 3: Key recent advances in paediatric blood and marrow transplantation

Intervention	Key advance	Elements / Exemplars	Limitation / challenges
Preparative regimens	Decreased short-and long term toxicity	1. Shift away radiotherapy- based conditioning in preparative protocols 2. Fludarabine as subsitute for as dose-sparing agent for Cyclphoshamide 3. refinements in Busulfan use, including intravenous dosing and pharmacokinetic monitoring 4. Non Busulfan-based regimens, e.g. use of Treosulfan	1. Restricted role for reduced-intensity preparative regimens in paediatric transplants (currently limited to aplastic anaemia and primary immunodeficencies)
Alternative donor transplants	Sucessful blood and marrow transplants from HLA-matched (or permissively HLA-mismatched) unrelated donors	1. Availability of high-resolution allele type of partinenet Class 1and Class II HLA loci 2. Alternative donor stem cell sources, including GCSF- mobilised peripheral blood and cord blood grafts 3. International network of accredited donor stem cell registries for volunteer donor recruitment, HLA-matching, stem cell collection and stem cell delivery 4. Use of serotherapy in preparative regimens to mltigate risk of GVHD	1. High cost ofacquiring unrelated donor grafts 2. Limited representation of defined ethnic groups in donor stem cell registries, limiting probability of identifying suitable HLA-matched donors for non Caucasian populations.
Supportive Care	Improvements in the prophylaxis and treatment of infections	1. Mould-active antifungal prophylaxis 2. Active surveillance for CMW infection and inervention with pre-emptive therapy 3. Similar surveillance for other herpes viruses (e.g. EBV) and adenoviruses	1. Emergence of multi-drug resistant bacterial 2. Absence of effective CMV prphylaxis

CMV : Cytomegalovirus : EBV : Ebstein-Barr virus - GCSF: Granulocyte colony stinulating factor; GVHD graft-versus-host disease

profound immunosuppression, thus facilitating stable donor haematopoietic stem cell engraftment while minimising the risk of GVHD. The dose-intensive nature of these regimens is associated with unique and potentially life-threatening acute organ toxicities, including idiopathic pneumonia and the hepatic sinusoidal obstruction syndromes. Radiotherapy- and alkylator-based preparative regimens are also associated with significant long-term complications, including endocrine morbidities (specifically growth failure and infertility) and second neoplasms (especially with radiotherapy-based regimens)(2). Preparative regimens have thus been modified to reduce these toxicities. Radiotherapy-based regimens are now largely confined to pBMT for acute lymphoblastic leukaemia. The introduction of Fludarabine, a relatively selective and potent lymphocyte toxin, has enabled dose reductions in Cyclophosphamide, especially in pBMT for constitutional marrow failure syndromes(3). Refinements in Busulfan use, including the move to the intravenous formulation and the introduction of pharmacokinetics-based dosing, have enabled optimal dosing within the drug's narrow therapeutic window(4). Recently, the Busulfan analogue Treosulfan has shown great promise, as it appears to have equivalent myeloablative and immunosuppressive potency, without the unpredictable pharmacokinetics and organ toxicity concerns of Busulfan(5). Concomitantly, interventions have been introduced to prevent or attenuate the short-term organ toxicity of preparative regimens, such as the introduction of Defibrotide in the prophylaxis and treatment of hepatic sinusoidal obstruction syndrome(6).

Unrelated Donor BMT

The availability of suitably matched unrelated donors has greatly widened access to pBMT treatment(7-9). High-resolution allele typing of pertinent Class I and Class II HLA loci have helped identify suitable HLA-matched unrelated volunteer donors. The lesser stringency for HLA matching of unrelated donor cord blood units has further expanded pBMT options for

Table 4 : HAPLOIDENTICAL, RELATED DONOR TRANSPLANTS STRATEGIES

	IA	IIA	IIIB	IB	III
STRATEGY	T-deplecte Megadose HPC no IS	T-replete G-primed BM+PBSC intensified IS	T-replete G-primed or PBSCPT-Cyclophosphamide	T-replete Selective T-depietion No IS	Hybirid graft Haplograft & ud-UCB Serotherapy as part of IS
MONIKER	The 'Perugia approach'	The 'Huang aproach'	The 'John Hopkin approach'	The 'Tubingen approach'	'Haplocord' trasplants
RATIONALE	Immune veto effect of megadose HPC	Composite G-primed grafts to ensure engraftment and lower GVHD risk	Preferential elimination of GVHD-mediating alloreactive T-cells	Elimination of alloreactive Tab and B lymphocytes	Heplodonor megadose HPC as bridge to support sustained late engraftment with single ud-UCB
ADVANGAGES	Engraftment GVHD risk No PT-ISI	Engraftment GCD	GVHD risk	Engraftment GVHD Immume recovery	Reduced-intensity conditioning Engraftment
DISADVANTAGES	Immune recovery GVD activity Techincal expertise	Acute GVHD Chromic GVHD	GVD Graft failure (non-malignant)	GVHD Graft failure (non-mailgnant) Technical expertise	Immune recover
REFINEMENTS	NK-alloreactive donor Donor Lymphocyte addback Tcon+Treg donor T-cell addback	Rapanrycin for GVHD prophylaxis Donor lymphocyte infusion	Ablative preparation regimen Donor lymphocyte imfusion recipient screening for DSAB 'Jefferson 2-step approach': ablative conditioning followed by a sequence of fixed dose donor T cell infusion, PTCy and HPC infusion	NK-alloreactive donore	

BM: Bone marrow, DSAB: Donor - specific Ab;G;-CSF (Granulocyte colony situmalating factory); GVHD: grafts-versus-Host Disease;GVD Graft-versus-Disease; IS: immuno suppresion; KIR; Killer Immunoglobullin Receptor, NK: Natural Killer Cell: Peripheral blood stem cells; PT-Cy: Post -Transplant Cyclophosphamider; PT-IS Post transplant immunosuppriession; TCON; conventional T-cell; Treg-Regulatory T cells; ud-UCB; unrelated donorumbilical cord blood.

Table 5: Unmetneeds and challenges in pBMT
1. Systemsmedicine approach to pBMT
Persionalised therapy based on intefration of variable such as:
(a) Host and donor gene polymorphisms that regulate innate and adaptive immune response
(b) The host microbiome
(c) Therapy variables (including gene polymorphisms that in fluence drug kinetics, response and tolerance)
(d) Graft variables (including source, use of mobilizing agents, cellular constituents and cell dose)
(e)Recipient disease status
(f)Recipient performance status and organ function
2. Rational management of GVHD
(a) Developing predictive and prognostic clinical and laboratiory biomarkers
(b) Tailored therapy based on targent organ in volvement and disease intensity
(c) Therapies that selectively target GVHD mediators
3. Solutions for resource-restricted economics
(a) Public funding based on recognition of pBMT treatment as public good
(b) National collaborative network of accredited well-resourced pBMT centres
(c)Accredited unrelated donor stem cell registry and unreleated cord blood bank linked to the pBMT network
(d) uniform standardized treatment protocols allowing systematic analysis and corss-referral arrangements
(e) Specialization of each pBMT centre in defined area to maxmise treatment options across the network
(f) Collaborative strategies to innovate and explore novel cost-effective treatment approached for patients
(g) Avoidance of resource duplication and concentration of expertise
4. Tackling the problem of infections with multidrug-resistant pathogens

patients. Multidisciplinary initiatives worldwide have resulted in the creation of an international network of accredited unrelated donor stem cell registries and repositories that oversee donor recruitment, perform haematopoietic stem cell collection and cryostorage and participate in donor identification and stem cell product delivery. Intensified immunosuppression through incorporation of lymphocyte-directed antibodies (anti-thymocyte globulin and the anti-CD52 monoclonal antibody alemtuzumab) in preparative regimens has helped address the risk of graft failure and acute and chronic GVHD associated with these transplants. Unrelated donor pBMT is however constrained by the considerable expense involved in acquiring unrelated donor products and the higher-risk of transplant-associated immune complications associated with unrelated-donor pBMT, including GVHD and infections.

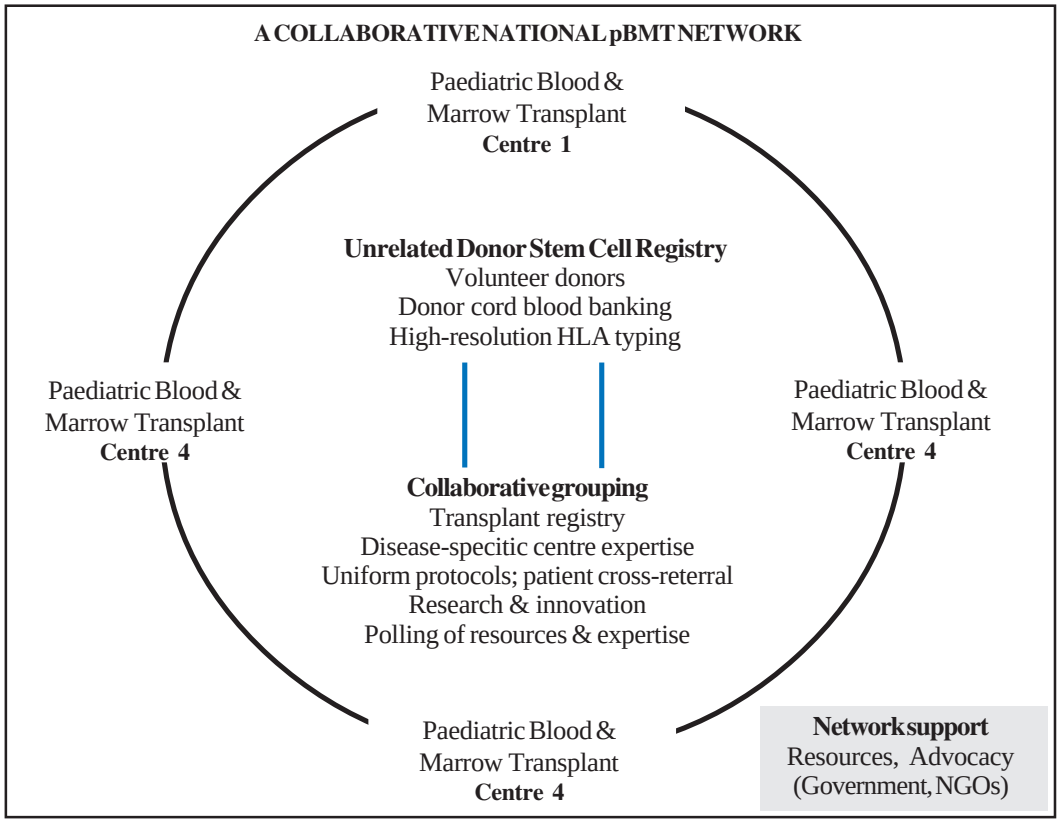
Advances in Infection Prophylaxis

A pivotal development in supportive care has been the introduction of infection prophylaxis measures(10). The profound immune paralysis associated with transplant therapy, especially in unrelated donor transplants where deeper immunosuppression is often required, has been associated with increased risk of invasive fungal and viral infections. This includes the pathogenic fungal moulds of the Aspergillus species and the Zygomycetes class as well as human herpes viruses (principally cytomegalovirus [CMV]), adenoviruses and the BK-JC polyomavirus. The introduction of mould-active antifungal prophylaxis and the strategy of pre- emptive intervention for CMV infection based on serial standardised monitoring of viral DNA copies, have significantly reduced the risk of invasive infections arising from these pathogens. Similar pre-emptive strategies have now been introduced for other viruses, including the Ebstein-Barr virus (especially with alemtuzumab-based regimens) and adenoviruses.

Evolving Advances

Haploidentical related donor pBMT: the potential of transplant for all: A significant advance in recent years is the increasingly successful outcome of haploidentical pBMT performed using first-degree family donors (parent or sibling)(11). This readily addresses limitations of donor availability and the high acquisition costs of unrelated donor stem cell products. Additionally, the availability of a family donor provides the option of post-transplant donor-derived cellular therapy. As described in Table 4, innovative approaches have been adopted to address the risks of GVHD and non-engraftment in haploidentical pBMT while ensuring timely haematopoeitic and immune recovery and maintaining graft-versus-disease (GVD) activity.

Extended therapeutic drug monitoring to inform drug use: Transplant outcomes are arguably greatly influenced by inter-patient variability in the pharmacokinetics and pharmacodynamics of transplant-associated medications. As with Busulfan and the calcineurin inhibitors, there is growing realisation of the need to extend drug monitoring to other agents commonly used in pBMT, such as Fludarabine, the antifungal triazoles and the immunosuppressive antimetabolite Mycophenolate. Results of such monitoring are expected to guide personalised dosing strategies12.



Novel and Next Generation Advances

Here, advances in two key areas are anticipated.

Expanded indications for pBMT treatments

This includes an expanded role for pBMT as regenerative and tissue repair therapy, exemplified by the remarkable experience with the genodermatoses(13). Additionally, pBMT treatment is likely to be increasingly used for long-term immune tolerisation, as in the case of simultaneous pBMT and solid organ transplantation. Here, immune tolerance as a consequence of pBMT obviates the need for long-term anti-rejection immunosuppression(14).

Cellular Therapies (Adoptive Immunotherapies)

These therapies involve administration of autologous or allogeneic haematopoeitic stem cell constituents without the use of immunosuppressive and preparative treatments. The antiviral efficacy of donor-derived virus-reactive cytotoxic T-cells is already well recognised. Similarly, partial HLA-matched alloreactive donor T-cells have the potential to temporarily engraft and mediate sustained cytoreduction of tumors(15). Autologous T-lymphocytes can be expanded in vitro and engineered to express tumor-directed receptors (called chimeric antigen receptor T-cells [CAR T cells]); early clinical experience with CAR T cell immunotherapy in B-lymphoid malignancies has been impressive16. In inherited monogenic disorders, autologous CD34- expressing stem cells can be modified ex vivo by retroviral transduction to restore gene function and the modified stem cells then reintroduced to correct disease phenotype, as successfully demonstrated in patients with X-linked severe combined immunodeficiency(17).

Unmet Needs and Challenges in pBMT Treatment

Despite these remarkable advances, there are significant lacunae in pBMT practice (highlighted in Table 5). A multitude of factors interact to influence outcomes in pBMT. This complex interplay requires a systems medicine approach that integrates these diverse variables. A rational management strategy is required to tackle GVHD, the scourge of pBMT treatment. This requires better biomarkers and more informed and sophisticated targeting of GVHD mediators(18).

Resource constraints mean that many modern and emerging therapies are beyond the reach of patients in resource-limited countries. Yet, transplant treatment represents the only curative option for many paediatric diseases. There is therefore a moral imperative to explore and develop new strategies in resource-constrained settings. One such model involves the development of a collaborative publicly funded national network of pBMT centres, details of which are outlined in Table 5 and presented in Figures 1 and 2.

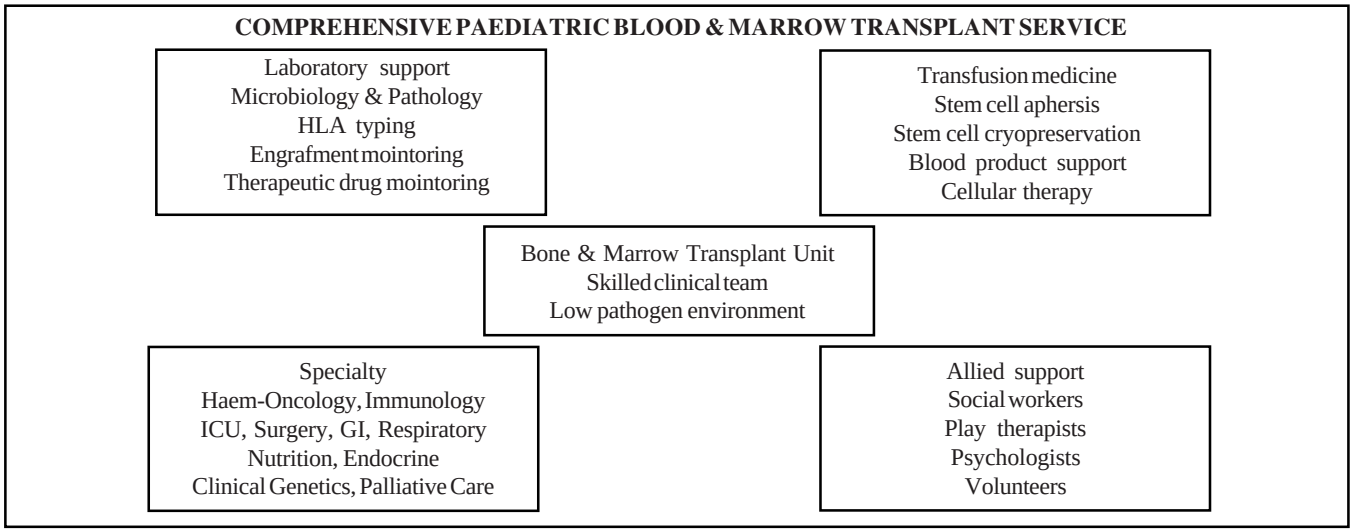


Fig 1: A collaborative national network of specialty multidisciplinary paedtric BMT centre

Concluding Remarks

These are exciting times in transplant medicine. There is now a real sense that the lofty goal of transplant for all eligible patients is within reach. Cost remains a major barrier and often provokes sharp questions about the role and value of pBMT treatment in countries that struggle with other more immediate healthcare priorities. Yet, as argued in Fig 2 and as observed by the Cure2Children Foundation in its pBMT initiative for transfusion-dependent thalassemia in South Asian countries(19), the practice of pBMT has the potential to catalyse sustained improvements in services across the continuum of paediatric healthcare and beyond. This appreciation of the wider salutary healthcare ramifications of pBMT should impel vigorous advocacy, enlightened public health policy and sustained investment.

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Figure 2: pBMT treatment (In this case, for genetic disease) catalyses multi-level continuum of healthcare

Early diagnosis	• Close supervision • Family counseling • Transplant assessments (donor availability, family commitment and resources)
Optimal pre-transplant care	• Minimise disease-related morbidity • Ensure satisfactory functional status pre-transplant
Timely transplant care	• Correction of disease phenotype • Minimise transplant-related complications
Early post-transplant care	Monitoring of • Graft function • Transplant-related complications • Immunosuppression therapy
Long-term post-transplant care	Monitoring of • Endocrine health • Second malignancies
Family screening	• Subsequent pregnancies in family • Extended family screening

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EMERGING SCENARIO

Proton Radiotherapy for Pediatric Rhabdomyosarcoma

A multicentric, prospective phase II study was performed to assess disease control with proton radiotherapy in children with rhabdomyosarcoma (RMS). Total 57 patients with localized RMS (age 21 years or younger) or metastatic embryonal RMS (age 2 to 10 years) were enrolled in the trial. All the patients were treated with proton radiation along with chemotherapy, either vincristine, actinomycin, and cyclophosphamide or vincristine, actinomycin, and ifosfamide-based chemotherapy. To assess and grade adverse effects of proton radiation, Common Terminology Criteria for Adverse Events, Version 3.0, was used. Results showed five-year event-free survival (69%) and overall survival (78%), similar to the trials that used photon radiation.. However the 5-year local control by risk group was 93% for low-risk and 77% for intermediate-risk disease and no acute or late toxicities higher than grade 3. Therefore proton radiation found to be safe and effective radiation modality for pediatric RMS.

(J Clin Oncol, Nov 2014)

Pediatric Adrenocortical Tumors

Adrenocortical tumors (ACT) in children are very rare and are most frequently diagnosed in the context of the Li-Fraumeni syndrome, a multiple cancer syndrome linked to germline mutations of the tumor suppressor gene TP53 with loss of heterozygosity in the tumors. Cancers associated with Li-Fraumeni syndrome include but are not limited to premenopausal breast cancer, bone and soft tissue sarcomas, acute leukemia, brain tumor, adrenocortical carcinoma, choroid plexus carcinoma, colon cancer or early onset of other adenocarcinomas or other childhood cancers. A peak of children ACT incidence is present in the states of southern Brazil, where they are linked to the high prevalence in the population of a specific TP53 mutation (R337H). Children ACT have specific features distinguishing them from adult tumors in their pathogenetic mechanisms, genomic profiles, and prognosis. Epidemiological and molecular evidence suggests that in most cases they are derived from the fetal adrenal.

(Front Endocrinol, Feb 2015)

PERSPECTIVE

EPIGENETICS IN CHILDHOOD LEUKEMIA

The haematological malignancies and among them the pediatric leukemia are the most common and aggressive forms of cancers in childhood. There is an improvement in the survival percentage of childhood leukemia (5-year survival among children 0-14 years of age: 89% lymphoid leukemia and 64% AML) due to the improved treatments. Leukemia is described as a genetic as well as an epigenetic disease. The disease stratification is an important component for effective treatment for balancing cure against toxicity. The identification of the nature of genetic lesion is essential, along with the new data accumulating on the epigenetic variations in leukemia, there is an attempt to generate reliable epigenetic markers that could be utilized for stratification in addition to the detection of chromosomal translocations.

In this brief write-up we attempt to discuss epigenetics as a phenomenon, its implication on the function of genes and provide a glimpse of the nature of studies that are being carried out in the area of epigenetics in leukemia.

What is Epigenetic Regulation

The term epigenetics was coined by Conrad Hal Waddington(1) in 1942 in an attempt to capture the lack of correlation between the genotype and the phenotype; individuals with identical genetic mutation fail to show concordance in disease symptoms or expression of the disease phenotype itself. The Greek prefix *epi* meaning "on the top of" implies that the epigenetic features override the effect of the nucleotide sequence of the gene. Epigenetics impacts gene expression without changing the nucleotide sequence of the gene and can be maintained through cell division. Presently epigenetic regulation occupies the central stage in the same context as was conceived by Waddington more than 70 years ago and is being explored for additional biomarkers for many diseases including leukemia. The major and well known molecular baseis of epigenetic regulation are, the post-replication modification of DNA and the post-translational modification of chromatin proteins, mainly the histones (Table 1). The addition of methyl group at the 5th position on the nucleotide base Cytosine leads to the formation of 5-methyl Cytosine (5mC). DNA methylation is correlated with down regulation or repression of gene expression. Therefore, hypermethylation of the promoter region of the tumor suppressor genes and hypomethylation of oncogenes can facilitate tumorogenesis. Therefore, just as loss-of-function mutation of tumor suppressor genes and gain-of-function mutation in oncogenes can occur in individual or group of cells, epigenetic alteration can also occur at the somatic cell level. Further, even in normal individuals, epigenetic variations can be detected not only between individuals, but also between tissues and as a function of time in terms of development and ageing of an organism. Epigenetics provides an interface between genetic constitution (genotype) of an organism and the environment.

Histone Modifications
Signature for active transcription
H2A Lys5 acetylation
H2B Lys5 acetylation
H2B Lys12 acetylation
H3 Lys4 acetylation
H3 Lys36 acetylation
H3 Lys9 acetylation
H3 Lys 14 acetylation
H4 Lys5 acetylation
H4 Lys16 acetylation
H4 Lys8 acetylation
H4 Lys12 acetylation
H3 Lys4 methylation
H3 Lys79 methylation
H3 lys27 acetylation
H3 Arg 17 methylation
H3 Thr11 Phosphorylation
H3 Lys36 methylation
H2B Lys 120 ubiquitylation
Signature for transcriptional repression
H2A lys119 monoubiquitylation
H3 Lys9 methylation
H4 Lys20 methylation
H3 Lys27 methylation

Table1. Histone modifications as signatures for gene expression. The most frequently observed post-translational modifications of histones are listed. The amino acid modified generally are Lysine (Lys/K) and Arginine (Arg/R). In literature, Histone H3 methylation is written as H3K27me, 27 stands for the position of the lysine residue in histone H3 protein. The number of methyl groups added can be one, two or three; H3K27me/H3K27me2/H3K27me3. H3K56 acetylation is correlated with DNA damage repair.

The post-translational modification of histones most often is the addition of acetyl, methyl or phosphate groups; though there are other more complex modifications like SUMOylation and ubiquitylation. Acetylation-deacetylation of histones can switch the expression of the gene from active transcription to repression, while the effect of methylation of histones can be either activation or repression depending upon the

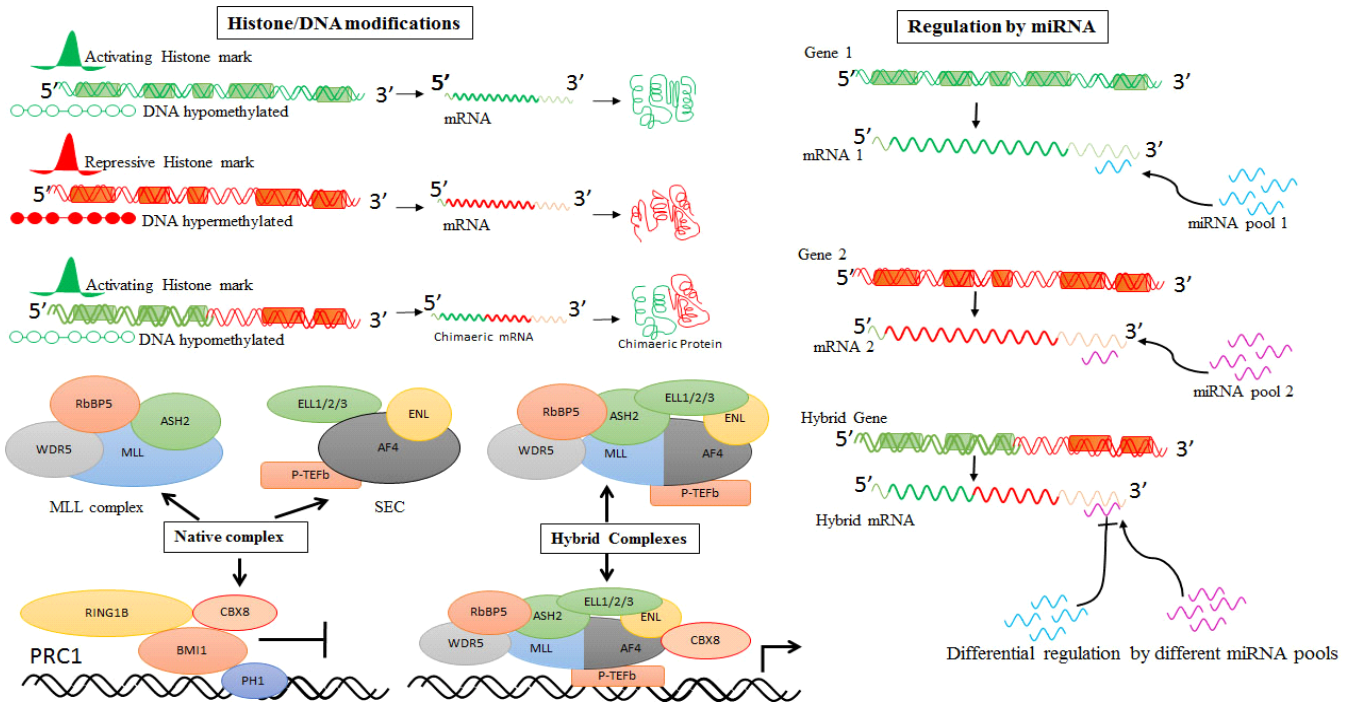

Figure1. A diagrammatic representation of the possible consequences of leukemic translocation on epigenetic regulation. The epigenetic marking at the 5' upstream region of the two translocation partners are depicted as a peak for histone modification or a chain of beads for DNA methylation. Among the various consequences, three consequences of leukemic translocation are shown. A- Translocationresults in bringing the 3'partner under activating epigenetic signatures instead of repression and gives rise to chimaeric protein, B- the chimaeric protein so formed forms hybrid complexes different from the native complex found in normal cells, C- the miRNA targeting for the translocation partners is altered due to translocation and the chimaeric mRNA produced. All the three altered epigenetic regulation lead to extensive changes in the gene expression profile of the cell compared to the normal cell, leading to leukemiogenesis.

histone and the specific residue of either lysine or arginine that is methylated (Table 1). Though epigenetic modifications may not be causative for disease phenotype, they are known to modify the disease symptoms in terms of expression and severity.

In addition to the covalent modification of DNA after replication and post-translational modification of proteins, there are other key epigenetic players like short and long non-coding RNA that regulate gene expression. The role of microRNAs in leukemia is very widely studied, though their implication in childhood leukemia is limited.

The DNA methylation and the histone modification are brought about by enzymes, such as the DNA methylases, histone methyltransferase and acetylases. Unlike the transcription factors that have sequence specific binding and thus, regulate specific genes, DNA methylases and histone modifiers bring about global changes in the gene expression pattern of a cell by affecting a large number of genes, conferring cellular identity through what is referred to as "cellular memory". It can be considered that cancerous state is nothing but a failed cellular memory programme, where the differentiated cell transits from the non-dividing state to an actively dividing state, one would expect that epigenetic alteration would be a major player in tumorogenesis.

DNA Methylation and Leukemia

The alteration in DNA methylation profile is known in several leukemic subtypes (2). There are several genes including HOX A4-A7, transporters(ABCB1), kinase genes(ABL1), apoptosis inducing gene(BIM), cell cycle control gene(CDKN1) that are detected to have aberrant DNA methylation in the promoter region leading to altered expression. It is interesting to note that regulatory RNA/microRNA coding genes also show variation in DNA methylation in their promoter region. The challenge is to decipher whether there are specific regions of altered DNA methylation that characterise a particular chromosomal translocation or leukemic subtype, so that it can be utilized as a potential biomarker for disease stratification and prognosis. Recently, the results of whole genome analysis of DNA methylation in a large cohort of B-lineage (137) and T-lineage (30) of ALL cases was reported (3). The authors identify epigenetic signatures common to all cases that negatively correlate with their expression level; higher DNA methylation with

lower expression and vice versa. Further, getting closer to generating a signature for stratification, they have identified distinct DNA methylation signatures for genetically distinct ALL subtypes.

If epigenetic modification is to be explored for possible therapeutic intervention, it is important to understand what triggers such a wide variation in DNA methylation. The enzymes that mediate DNA methylation, DNA methyl-transferases are known to have increased expression in certain leukemia, but not all of them. There are therapeutic strategies involving the use of non-methylatable analogues of Cytosine, such as 5-azacytidine (Vidaza®) and 5-aza-2'-deoxycytadine(Dacogen®) in clinical use, but no inhibitors of DNA methylases are known so far.

Histone Modification and Leukemia

In comparison to DNA methylation in ALL, histone modification is less studied presently especially from the angle of leukemic subtype characterization. However, the mutations in genes coding for epigenetic modifiers are common in leukemia including ALL and are one of the promising areas of investigation. One of the examples of translocations including epigenetic modifiers is t(4:11) involving MLL(mixed lineage leukemia) and t(4:14) involving NSD2(nuclear receptor binding SET domain protein 2). MLL is the human homologue of the Drosophila trithorax protein and its biochemical function is the methylation of histone H3 at the lys 4 residue (H3K4me3) which opens up the chromatin for transcriptional activation. The consequence of this covalent modification of histones is to maintain active state of expression of genes that are targets of MLL. The MLL protein forms a complex with other proteins, some of them having DNA binding property and the ability to open up the tightly packaged chromatin, referred to as chromatin remodelling proteins. When there is unholy joining of a part of the MLL gene with another gene forming a hybrid protein, as in leukemic translocations, the target specificity for histone modification and hence for maintaining active state of transcription is completely altered. This is one type of epigenetic invasion of the genome that is possible in ALL. There are several examples of such fusion in leukemia, as discussed below.

In case of MLL-AF10 translocation, the fusion protein interacts with H3K79 methyltransferase (Dot1L) to bring about an increase in H3K79me2 at various *HOX* genes leading to an increase in transcription (4). MLL-AF9 leukemogenesis is dependent on the presence of the native MLL on the normal (untranslocated) homologue, specifically for the proliferation of the leukemic cells. The high level of expression of different *HOX* genes in leukemia could be the consequence of altered DNA methylation and the presence of activating histone marks due to the recruitment of the MLL-AF9 fusion protein as well as native MLL complex at the *HOXA9* locus. The native MLL complex maintains the high level of H3K4 methylation and is also necessary for H3K79 methylation(5). In addition, the MLL fusion proteins formed as a result of translocation can bring about changes in the composition of native complexes formed by either of the partners as compared to their fusion product. Hybrid complexes inappropriately combine the properties of two individual complexes, thus leading to functional aberration. For example, in case of t(4;11)(MLL-AF4) and t(11;19) (MLL-ENL),MLL fusion with either AF4(sequence specific DNA binding function) or ENL(suppressor of RNA polymerase pausing), leads to the generation of hybrid complexes that are recruited to MLL target loci and thus bring about sustained activated transcription of MLL targets leading to leukemiogenesis (6). AF4 and ENL are members of the SEC (Super Elongation Complex), related to AEP or PAF complexes, that bring about activation of transcription. In yet another example, leukemic cells with MLL-AF9 fusion, CBX8 (Chromobox homologue 8) acts as a transcriptional co-activator, whereas, in normal cells CBX8 is a member of PRC1 (Polycomb Repressive Complex 1), that brings about transcriptional repression. This role reversal supports aberrant activation of MLL-AF9 target genes(7).

It is also interesting to consider epigenetic factors in facilitating chromosomal translocations. MLL-rearranged translocations occur due to the presence of mutational hot-spot known as the breakpoint-cluster region (BCR) within the MLL gene. The chromatin architecture in and around the BCRs can affect the transcription of fusion product resulting from chromosomal translocations. It further remains to be elucidated whether the differential chromatin environment characterises a hotspot for breakpoint and hence translocations. MLL BCR contains accessible or active chromatin due to the presence of increased H4 acetylation and reduced H3 acetylation. H1 histone is further found to be asymmetrically distributed, being high at the centromeric and low at the telomeric ends (8). The M-bcr which involves the Philadelphia chromosome translocation, contains a sub-region of around 600bp

region that is hypomethylated, However, about 40% of CML patients, show aberrantly hypermethylated M-bcr, although it was established that translocation was not responsible for the hypermethylation (9).

Another dimension to epigenetic regulation is added by the microRNA (small non-coding RNAs; 18-23 nucleotide long). miRNAs are responsible for the fine tuning of gene expression as they can either lead to degradation of mRNA or bring about translation inhibition. miRNA-203 decreases the levels of ABL1 and BCR-ABL1 fusion protein. In CML and ALL, the region upstream of this microRNA is hypermethylated leading to low level of miRNA-203, hence high concentration of the fusion protein. The epigenetic drugs such as 5'- azacytidine (Aza) and 4-phenylbutyrate or their combination, led to the demethylation, which resulted in a dramatic increase in miR-203 levels which in turn brought down the ABL1 and BCR-ABL1 protein levels. This led to the reduction in proliferation of the CML cell lines, K562 and KCL-22 (10), As a corollary, the overexpression of a miRNAcan reduce leukemic cell proliferationas in the case of miRNA-150 (11). The miR-150, a tumor suppressor microRNA, is known to be repressed in acute leukemia and MLL-fusion proteins are implicated in this repression.

Epigenetic Drugs

It is well established that leukemia is a genetic as well as an epigenetic disease. Thus, drugs involving those that alter the chromatin environment may be effective as monotherapy or as a combinatorial chemotherapy. A well-known class of the "epigenetic drugs" is the histone deacetylase inhibitors (HDACi). One of the most popular HDACi is the pan-HDAC inhibitor Panobinostat, sold as an oral drug under the trade name of Farydak/ Faridak. Panobinostat has been used against various kinds of haematological malignancies. A number of HDACi are still under clinical trials. These include SAHA (suberoylanilideHydroxamic acid), Valproic acid, MS-275, depsipeptide and phenylbutyrate. Studies involving a number of these epigenetics drugs in combination with other drugs have been completed. SAHA in combination with fludarabine/cloflarabine/busulfan is being used in clinical trials for the treatment of Acute Leukemia (Clinicatrials.gov identifier :NCT02083250). Another ongoing study uses MS-275 with Azacytidine for the treatment of myelodysplastic syndromes, Chronic myelomonocyticleukemia, or acute Myeloid leukemia (clinicaltrials.gov identifier :NCT00101179). A recently completed clinical trial used valproic acid in combination with decitabine in treatment of patients with refractory or relapsed acute myeloid leukemia along with previously treated lymphocytic leukemia (clinicaltrials.gov identifier : NCT00079378). MS275 and GM-CSF have been used in combination in a recently completed study for the treatment of myelodysplastic syndromes and refractory or relapsed acute myeloid leukemia or acute lymphocytic leukemia (clinicaltrials.gov identifier :NCT00462605).

In summary, attacking childhood leukemia through the modulation of epigenetic regulation is a promising future direction of treatment by combinatorial approaches. The generation of the data on epigenetic signature for distinguishing leukemic subtypes is essential to develop effective therapeutic strategies.

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IN FOCUS

MINIMAL RESIDUAL DISEASE IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

Introduction

Over the past three decades remarkable progress has been achieved in the treatment of acute lymphoblastic leukemia in children. Current treatment strategies result in long term remission for nearly 80% of children with ALL, the remaining 20% ultimately relapse and cure rate after relapse is approximately 25-40%. Relapses are caused by residual malignant cells that are undetectable by standard diagnostic techniques.

The response to treatment in patients with leukemia traditionally has been assessed by morphological assessment of blast percentage in peripheral smear and bone marrow aspirate smears. This traditional approach is subjective and quite limited in sensitivity. To be detected with certainty, the leukemic blast cells must constitute at least 5% of the total nucleated cell population [1]. With contemporary chemotherapy regimens, only a few patients with ALL have unusually high percentages of marrow leukemic lymphoblasts persisting during remission induction therapy. Thus, a patient declared to be in complete clinical remission may, in fact, harbor as many as 10^{10} leukemic cells.

The morphological assessment of remission is quite challenging when leukemic cells are present in small numbers. This is particularly true in patients with acute lymphoblastic leukemia (ALL), because the morphology of ALL blast cells is often indistinguishable from that of lymphoid precursors (the progenitors of B-lymphocytes, often called hematogones by hemopathologists) and activated mature lymphocytes and this is particularly true for post chemotherapy and post transplant bone marrows where the percentage of hematogones may surpass 10% of the total cellular population.

Technological advancement has led to introduction of the concept of minimal residual disease ie detecting residual disease with techniques more sensitive than morphology alone. Concept of minimal residual disease has challenged the conventional definition of "remission" and has proven over time to have an independent clinical relevance [2,3,4,5].

Prognostic Significance of Minimal Residual Disease in Childhood Acute Lymphoblastic Leukemia

One of the most immediately obvious applications of MRD testing is its use in measuring early treatment response and identifying patients who achieve morphologic remission but still harbor considerable levels of disease. The prognostic value of such tests in childhood ALL was demonstrated most convincingly by three large prospective studies reported in the late 1990s by the European Organisation for Research and Treatment of Cancer (EORTC)[6], St Jude [7], and BFM [8], groups.

These and others unequivocally demonstrated that MRD detected during the first 2 to 3 months of therapy is the strongest predictor of relapse. MRD also can help identify patients with a higher risk of relapse among those with specific ALL subtypes, and among patients with first-relapse ALL who achieve a second remission, and patients with isolated extramedullary relapse. Detection of MRD before allogeneic hematopoietic stem cell transplantation (HSCT) is associated with an increased risk of relapse after HSCT [9,10,11].

Although the clinical significance of MRD is now clear, initial efforts to systematically study MRD in patients were met with some skepticism about the clinical value of MRD testing. This often stemmed from the belief that leukemia distribution might be extremely heterogeneous, rendering MRD testing uninformative in regards to residual leukemic burden and treatment response. Others thought that MRD studies might not provide any additional information over established clinicobiologic prognostic features of ALL [12,13].

Numerous studies now have demonstrated conclusively that MRD is a powerful prognostic indicator in childhood ALL, and there is mounting evidence that this is also the case in adult ALL patients. Therefore, an increasing number of treatment protocols use MRD measurements for ALL risk assignment.

A commonly used cut-off level to define MRD positivity is 0.01% of bone marrow mononuclear cells. Selection of this level is due to the fact that this is the typical limit of detection for routine flow cytometric and molecular assays. It is shown to discriminate between patients with different risks of relapse. Studies show that patients who had MRD of 0.01% or higher in bone marrow at any treatment interval monitored had much higher risk of relapse in several studies [7,11,14].

Implications in Treatment Decisions and Risk Stratification

Various risk stratification models incorporate MRD measurement at different time points as a necessary investigation.

Berlin-Frankfurt-Münster (BFM) risk groups

Since 2000, risk stratification on BFM protocols has been based almost solely on treatment response criteria. In addition to prednisone prophase response, treatment response is assessed via MRD measurements at two time points, end induction (week 5) and end consolidation (week 12)[15].

The BFM risk groups include the following:

- *Standard Risk:* Patients who are MRD-negative (i.e., $<10^{-4}$) at both time points are classified as standard risk.
- *Intermediate risk:* Patients who have positive MRD at week 5 and low MRD ($<10^{3}$) at week 12 are considered intermediate risk.
- *High risk:* Patients with high MRD ($\geq 10^{-3}$) at week 12 are high risk. Patients with a poor response to the prednisone prophase are also considered high risk, regardless of subsequent MRD.

Treatment Intensity is Regulated Accordingly

At St. Jude Children's Research Hospital, the Total 16 study used MRD levels on day 15 and day 42 for treatment assignment. Patients with MRD of greater than or equal to 1% on day 15 receive intensified remission induction therapy; further intensification is reserved for patients with greater than or equal to 5% leukemic cells. On the other hand, patients with MRD less than 0.01% on day 15 receive a slightly less intensive reinduction therapy and lower cumulative doses of anthracycline. Patients with standard-risk ALL who have MRD of greater than or equal to 0.01% on day 42, are reclassified as high-risk; patients with MRD greater than or equal to 1% are eligible for HSCT in first remission.

COG protocol AALL08B1 stratifies four risk groups for patients with B-precursor ALL (low risk, average risk, high risk, and very high risk) based on the following criteria:

- Age and presenting leukocyte count (using NCI risk-group criteria.
- Extramedullary disease (presence or absence of CNS and/or testicular leukemia).
- Genomic alterations in leukemia cells.
- Day 8 peripheral blood MRD.
- Day 29 bone marrow morphologic response and MRD.
- Down syndrome.
- Steroid pretreatment.

Assessment of Minimal Residual Disease by Flow Cytometry

ALL cells express immunophenotypic features that can be used to distinguish them from normal hematopoietic cells, including hematogones and activated lymphocytes commonly referred to as Leukemia associated immunophenotype (LAIP).

Table 1 : Risk Groups for B-Precursor Acute Lymphoblastic Leukemia[a]

	Low Risk	Average Risk		High Risk			Very High Risk			
NCI Risk (Age/WBC)	SR	SR	SR	SR	SR	HR(age<13y)	SR	HR	HR (age≥13y)	SR or HR
Favorable Genetics	Yes	Yes	Yes							
Unfavorable Characteristics	None	None	None	None	None	None	None	None	None	None
Day 8 PB MRD	<0.01	≥0.01%	<1%	Any Level	≥1%	Any Level	Any Level	Any Level	Any Level	Any Level
Day 29 Marrow MRD	<0.01 %	<0.01%	<0.01%	≥0.01%	<0.01%	<0.01%	≥0.01%	≥0.01%	<0.01%	
% of patients (Estimated)	15%	36%		25%			24%			
Anticipated 5-year EFS	>95%	90%-95%		88%-90%			<80%			

EFS=event-free survival;HR=age adn WBC count risk groupi high risk, MRD=minimal residual disease, NCI=National Cancer Institute; PB=peripheral blood;SR=age/WBC count risk group is standard risk; WBC=white blood cell

In virtually all patients with ALL, leukemia-associated immunophenotypes can be defined at diagnosis and then used to monitor MRD during treatment. Immunophenotypes sufficiently dissimilar from those of normal cells to allow a sensitivity of detection of 0.01% are expressed by most cells in approximately 95% of cases [14,16].

The reliability of flow cytometric MRD assays depends on several factors, the most important ones being the correct marker combination in use, adequate number of cells should be available for the study[17]. Finally, and perhaps most importantly, the laboratory performing the studies must have specific expertise in MRD assays. Simple availability of a flow cytometer and experience in leukemia immunophenotyping are not sufficient to perform MRD studies proficiently.

Summary

It is unquestionable that MRD tests allow leukemia remission to be defined in a way that is much more accurate and rigorous than the one afforded by conventional morphologic techniques. In addition to their capacity to predict outcome on the basis of early response to therapy, MRD methods also can be used to recognize leukemia relapse before it is morphologically overt, to determine the leukemia burden before HSCT, and to measure the efficacy of a treatment regimen in relation to that of its predecessor.

MRD assays are complex and require expertise to be performed well. Although MRD testing is relatively expensive compared with other routine laboratory assays performed at diagnosis in patients with ALL, it provides unique and powerful information that should not only improve treatment but, in the long run, also reduce overall clinical management costs[18].

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(Dr Sonal Jain, Consultant, Dept of Pathology)

RECENT ADVANCES

ADVANCES IN NUCLEAR MEDICINE IN PEDIATRIC ONCOLOGY

Pediatric Nuclear Medicine is a very exciting, dynamic and growing field. An important advantage of nuclear medicine procedure is that the radiopharmaceuticals used are non toxic, target specific and require easily customized procedure to reduce radiation burden. Physiological and molecular information generated by nuclear medicine tests are incomparable. Pertaining to pediatric nuclear oncology, the field has seen development of various radiopharmaceuticals in both diagnostic and therapeutic. 131-Iodine, ^{131}I-MIBG, 99m-technetium (^{99m}Tc) labeled radiopharmaceuticals are time tested. Major development in nuclear medicine has occurred due to invention of positron emission tomography-computed tomography (PET-CT). PET-CT is a revolutionary technique in which various biological molecules (glucose, amino acid, peptide, antibodies, DNA nucleobase etc.) are used to target physiological and pathological process. ^{18}F- Fluoro-deoxy-glucose (FDG), ^{68}Ga-DOTATATE, DOTANOC, DOTATOC, ^{18}F-DOPA, ^{18}F-5HTP, ^{18}F-FLT, ^{18}F-FET, ^{11}C-Methionine, ^{177}Lu-DOTATATE, ^{131}I-Tositumomab (Bexxar) and ^{90}Y-Ibritumomab (Zevalin) are the few popular ones. Some of these are presented here.

^{18}F- Fluoro-deoxy-glucose (FDG) PET-CT

F-18 FDG PET-CT scan is the most frequently used nuclear medicine procedure in current pediatric oncology practice. FDG works on the principle that cancer cells have increased glucose turnover due to high expression of GLUT-1 receptor and increased glycolytic activity. More frequently indications for FDG PET-CT in pediatric oncology are given in Box 1. Less frequent indications for PET imaging in pediatric oncology include evaluation of germ cell tumors, hepatoblastoma, Wilms tumor and neurofibromatosis type 1 for suspected malignant transformation of neurofibroma.

Somatostatin Receptor Scintigraphy (SRS)

Over expression of somatostatin receptor (most common SSTR2 subtype) in neuroendocrine tumor is the fundamental for exploiting this imaging. ^{111}In-pentriotide was first FDA approved SRS tracer used in Gamma camera. Due to better image quality and wide spread availability, PET-CT based somatostatin receptor scintigraphy (SRS) has become the current choice. ^{68}Ga labeled somatostatin analogs, i.e. DOTATOC, DOTATATE and DOTANOC are the most commonly used so far. DOTANOC, is preferred due to wider spectrum (SSTR2, 3 & 5). SRS is frequently used in neuroendocrine tumors to look for unknown primary site, staging, recurrence evaluation and planning peptide receptor radionuclide therapy (PRRT).

Neuroblastoma and primary brain tumor (medulloblastomas and supratentorial PNET) are the other pediatric tumors expressing somatostatin receptors. SSTR binding has been seen in 80-90% of neuroblastoma.

Limited data is available on use of SRS in pheochromocytomas (PCC) or paragangliomas (PGLs). SRS has reported better imaging in aggressive PGLs. Only 2/3rd of insulinomas expressed somatostatin receptor 2 and 5, while non malignant insulinomas rarely expressed SSTRs. SRS is more sensitive detection and staging of malignant insulinomas because of dense over expression of SSTRs.

^{18}F-FDOPA PET/CT

Dihydroxyphenylalanine (DOPA) is the precursor of all endogenous catecholamines and taken up by amino acid transporter to cytoplasm and converted to dopamine (DA) by decarboxylase enzyme. FDOPA was first exploited for neurological diseases but it was soon realized, that being an amino acid marker it can be used for tumor imaging as well. Reported sensitivity and specificity were 79% and 95% respectively for PCC/PGLs. It has no uptake in normal adrenals so can be used for suspected adrenal hyperplasia. FDOPA PET CT is excellent for head & neck PGLs with sensitivity for reaching to 100%. Its sensitivity is similar to MIBG for

Box 1. Common indications of FDG PET CT in current pediatric oncology

A.	Lymphoma (HD und NHL): Staging, response to therapy, restaging, assessment of residual masses after therapy, planning of radiation therapy.
B.	Musculoskeletal malignancies (osteosarcoma, Ewing's sarcoma and soft tissue sarcoma, in particular rhabdomyosarcoma): staging, response to therapy, restaging/detection of relape.
C.	Neuroblastoma (generally in MIBG-negative cases).
D.	CNS tumors (grading, prognostic stratification, response to therapy, detection of recurrence, radiation therapy planning.)
E.	Carcinoma of unknown primary site (CUPS).

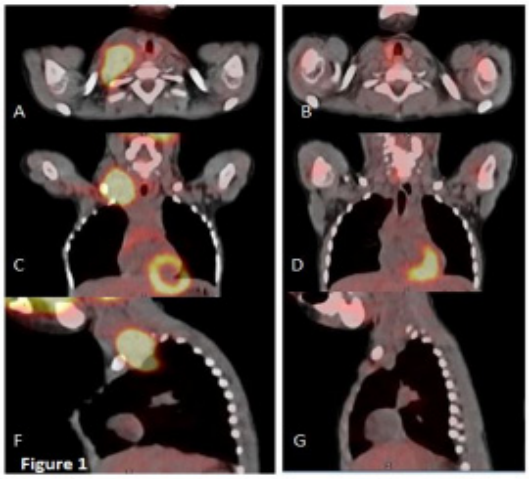

Figure 1: FDG PET-CT fused axial (A, B), coronal(C, D) and sagittal (F, G) images. Hodgkin's lymphoma patient showed metabolically active right cervical lymphadenopathy (image A, C, F). Complete response is seen in post chemotherapy scan (image B, D, G).

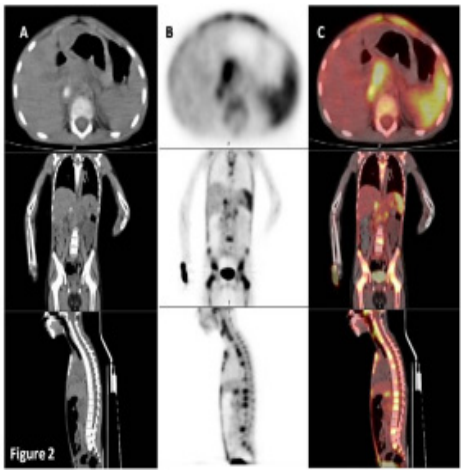

Figure 2: CT (A), FDG PET (B) and fused (C) axial, coronal and sagittal images in a suspected case of neuroblastoma. FDG PET CT revealed metabolically active partially calcified right supra renal mass with retroperitoneal lymphadenopathy and multiple bony metastasis.

PCC. FDOPA has excellent outcome in multifocal PGLs and metastatic PGLs with unknown genetic status. 18-FDOPA has reported good PET tracer for localizing insulinomas and focal beta cell hyperplasia with sensitivities varying from 75% to 100%.

^{11}C-5-HTP PET/CT

5-hydroxytryptophan (5-HTP) is a serotonin precursor. ^{11}C-5-HTP PET has showed better sensitivity than 18F-FDOPA and SRS for pancreatic NETs. It has been postulated that islets cell tumor has more serotonin activity than other NETs. 11-carbon (^{11}C) has a very short half life ($T_{1/2}$: 20 minutes). On site cyclotron is required for this. Moreover, the difficult synthesis process of ^{11}C-5-HTP restricts its use globally.

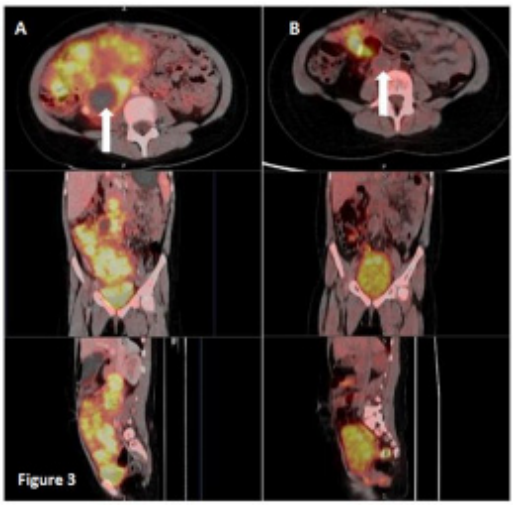

Figure 3: Fused FDG PET-CT axial, coronal and sagittal images in a case of right ovarian dysgerminoma. Pretherapy scan (images A) revealed metabolically active avid right ovarian mass with metabolically active avid necrotic retroperitoneal lymphadenopathy. Post therapy (surgery with chemotherapy) scan (images B) revealed metabolically inactive necrotic paracaval lymph node (white block arrow) suggests good response to treatment.

^{18}F-Fluoro-Ethyle-Tyrosine (FET)

Brain tumors are the most common solid tumors in children and mostly are primary. The most common types of brain tumors in children are astrocytoma, medulloblastoma and ependymoma. MRI is the investigation of choice for primary assessment. But many a times it is difficult to qualify a ring enhancing lesion on MRI as tumor (glioma, metastasis or other primary brain tumor) or non tumor (abscess, parasite, demylination, infarct or old hematoma) origin. Other issue of tumor recurrence in post surgery or radiotherapy setting is very troublesome. 18F-fluoro-ethyle-tyrosine (18F-FET) is an artificial amino acid and taken up by up regulated tumor cells. 18F-FET scan has proved its efficacy in

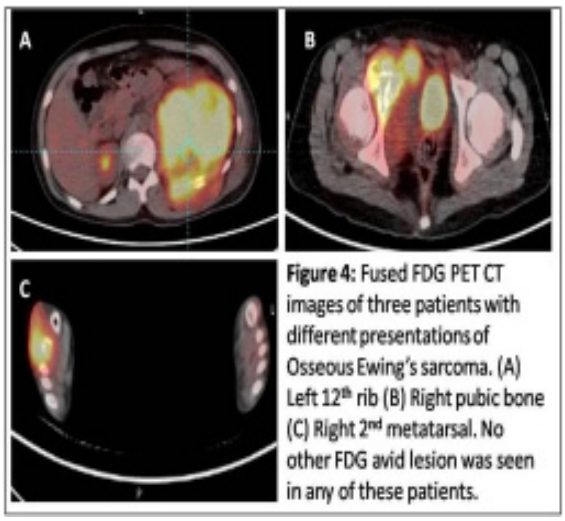

Figure 4: Fused FDG PET CT images of three patients with different presentations of Osseous Ewing's sarcoma. (A) Left 12th rib (B) Right pubic bone (C) Right 2nd metatarsal. No other FDG avid lesion was seen in any of these patients.

brain tumor recurrence after surgery and radiotherapy (Fig. 5). 18F-FET shows lower uptake in inflammatory cells than 18F-FDG, so it can be utilized in this diagnostic dilemma of tumor Vs inflammation. In other potential uses, it can be used for directing biopsy and radio-surgery.

Radioimmunotherapy

Radioimmunotherapy (RIT) is a cancer treatment in which a combination of radionuclide (source of radiation) and monoclonal antibody is used. Antibodies are the part of body immune system that recognize foreign antigen (bacteria and virus) and activate immune system to kill them. Scientist now can develop specific antibodies to target specific antigen that is present on specific cancer cell. After these antibodies injected into bloodstream, they travel throughout the body and attach themselves to specific target antigens on cancer cells. This process alerts the body's immune system and helps in destroying the cancer cells. During this process some normal cells that also have the specific target antigen will also be attacked. However, the body usually replaces these normal cells following treatment. In the RIT, besides activating immune system, radioactive particle also delivers a lethal dose of radiation to cancer cell.

Two RIT products are currently US Food and Drug Administration (FDA) approved for patients with B-cell lymphomas: Yttrium-90 ibritumomab tiuxetan (Zevalin) and Iodine-131 tositumomab (Bexxar). Both antibodies recognize a specific antigen called "CD20" on the surface of cancer cells in patients with B-cell non-Hodgkin lymphoma (NHL). These treatments are generally well tolerated without hair fall or serious side effects associated with chemotherapy. Most common side effect of these is low blood counts (occurring between 4 to 8 weeks following treatment). Weekly blood count for this period is to be monitored to assess bone marrow suppression following these therapies. Additional side effects include weakness, fever, nausea, infection, cough, or occasional severe allergic reaction.

RIT is currently under clinical trial and used for relapsed (disease returning after treatment) or refractory (disease not responding to treatment) follicular lymphoma or other indolent (slow-growing) lymphomas as well as previously treated follicular lymphoma patients who achieve partial or complete response to chemotherapy.

Peptide Receptor Radionuclide Therapy

Peptide receptor radionuclide therapy (PRRT) commonly uses a therapeutic radionuclide (^{177}Lu) labeled peptide (somatostatin) to target specific receptor which over expresses in a cancer cell. Most commonly used PRRT agents are ^{177}Lu-DOTATATE and ^{90}Y-DOTATOC. As already has been discussed in SRS neuroendocrine tumor over expresses somatostatin receptor (most commonly SSTR2). Somatostatin is the standard treatment in non operable neuroendocrine tumor. DOTATOC or TATE are analog of somatostatin and

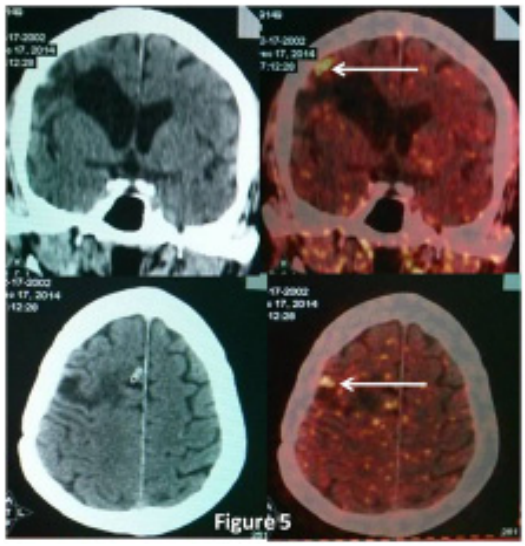

Figure 5: CT and Fused 18F-FET PET-CT axial and coronal images of brain: FET positive small focus (white arrow) along superior surface of post operative cavity in right fronto-parietal region in grade 2 oligodendroglioma.

produce the same effect. In addition, labeled radionuclide will give the lethal dose of radiation to cancer cell and produce stronger effects.

Currently these agents are used in clinical trials for SSTR2 postive grade 1 or 2 neuroendocrine tumors. Patients with pheochromocytomas, paragangliomas, neuroblastoma and medullary carcinoma thyroid may also be included. The prerequisite for PRRT is a positive ^{68}Ga-DOTANOC scan. A German multi-institutional registry study with prospective follow up in 450 patients indicates that PRRT is an effective therapy for patients with G1-2 neuroendocrine tumors, irrespective of previous therapies, with a survival advantage of several years compared to other therapies and only minor side effects. Median overall survival of all patients from start of treatment was 59 months. Median progression-free survival (PFS) accounted to 41 months. Grade 3-4 nephro- or hematotoxicity were observed in only 0.2% and 2% of patients, respectively.

Baum RP. et al have also reported that none of their patients (n-24) showed grade 3 or 4 nephrotoxicity and PRRT resulted in partial remission in 36% and stable disease in 36% of the patients, and 28% had progressive disease. From RGCIs perspective the facilities like FDG PET-CT & Somatostatin receptor scintigraphy are availabe routinely and the protocols are in place for PRRT. Newer pharmaceuticals have come to the bed side notably the FET for brain tumors and few are in the pipeline

(Dr. Manoj Gupta, Consultant; Dr. P.S. Choudhury, Director of Nuclear Medicine)

PAPERS PUBLISHED BY RGCI&RC 2014: ABSTRACTS

Gupta A, Kapoor G, Jain S, Bajpai R.

Absolute Lymphocyte Count Recovery Independently Predicts Outcome in Childhood Acute Lymphoblastic Leukemia: Experience From a Tertiary Care Center of a Developing Country

J Pediatr Hematol Oncol

Background

Acute risk stratification is essential for successful treatment outcome in childhood acute lymphoblastic leukemia. Early recovery of absolute lymphocyte count (ALC) during induction therapy is emerging as a reliable and favorable prognostic indicator that may hold its relevance in resource-constraint settings.

Materials and Methods

This is a retrospective chart review of medical records of 212 patients of acute lymphoblastic leukemia, aged less than 18 years, treated between January 1996 and December 2009. Time to lymphocyte recovery was analyzed with respect to various prognostic factors and survival and Martingale residuals were used to define ALC cut-offs.

Results

High-risk disease characteristics include older age (10 Y and older), high risk, and central nervous system diseases at diagnosis were associated with delayed lymphocyte recovery. The 5-year event-free, relapse-free, and overall survival of patients with day 15 ALC of e" 500 cells/μL and day 29 ALC of e" 1000 cells/ μL was 81.7% ± 4%, 86.4% ± 2.8%, 91.0% + 3%, respectively, Those with delayed recovery were (16.6% ± 5.6%, 19.3% ± 6.4%, 32.8% ± 7.2%, P < 0.001). In multivariate analysis both these ALC cut-offs retained their significance as prognostic variables of survival.

Conclusions

Our analysis revealed ALC to be an important independent predictor of treatment outcome and may provide key prognostic information in settings where minimal residual disease-based risk stratification is not feasible.

Dhamija M1, Kapoor G, Juneja A.

Infusional Chemotherapy and Medication Errors in a Tertiary Care Pediatric Cancer Unit in a Resource-Limited Setting

J Pediatr Hematol Oncol. 2014 Oct

Background

Drug administration is a multiprofessional process. The high toxicity and low therapeutic index of chemotherapy drugs make medication errors a significant problem, resulting in excessive patient morbidity and cost.

Objective

An audit of the delivery of infusional chemotherapy among pediatric inpatients was planned, with the objective of improving practice and minimizing errors.

Method

An observational study was conducted between January and August 2012. Patients were followed up from their premedication until the completion of post chemotherapy hydration and/or rescue drugs. Errors were recorded and classified by error type, cause, severity, unit location, medication involved, and harm caused.

Results

A total of 205 observations were made and 23(13.6%) errors recorded, of which 6 were intercepted. No life-threatening adverse drug event was recorded. The most important risk factor predisposing to errors was admission to nonpediatric ward (P=0.004). Documentation errors and incorrect infusion time were the two most common errors, whereas the most frequent error node was administration error. Appropriate steps were taken to prevent their reoccurrence.

Conclusions

This study helped provide important information about the rate and epidemiology of medication errors, emphasizing on the role of audit in enabling development of appropriate error-reducing strategies, particularly in the context of quality assurance in hospitals.

Rajiv Gandhi Cancer Institute and Research Centre

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- Stereotactic Radio Therapy (SRT)
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