Unravelling the ‘known unknowns’: lessons and reflections from the New Directions in Leukemia Research 2012 Conference

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Abstract

Patients diagnosed with leukemia approach their treatment with the hope of cure despite the effect on their quality of life. Some patients will be cured, others will die from treatment and some will die of their disease. A common theme at The New Directions in Leukemia Research (NDLR 2012) meeting was that cure will come if the drivers of the disease are better understood. Key messages were the power of combination platforms to understand the genetic and epigenetic modifications in leukemia to enable development of rational therapies, which can be tested via new clinical trial designs ensuring rapid clinical implementation.

Statement of purpose

NDLR is the only biennial international conference focused on leukemia. The aims of the meetings are to bring together scientists and clinicians to discuss and debate current concepts in our understanding of the molecular basis of leukemia, emerging paradigms and breakthroughs at the forefronts of leukemia research, and new therapies emerging in the clinic. There were 8 international speakers, 10 national speakers, 17 selected oral presentations and 53 posters. The meeting also hosted an early career forum, sponsored by the Leukemia Foundation of Australia, where some of the international and national speakers shared the trials and tribulations of their journey in science with 60 early career researchers.

Success in CML and why current targeted therapies don’t suit everybody

Tim Hughes (Centre for Cancer Biology, Adelaide, Australia) gave the NDLR 2012 Special Oration reflecting on what imatinib has taught us about chronic myeloid leukemia (CML) and targeted
therapy. He discussed the advancements that have enabled CML to change from an invariably fatal disease to a disease where the 5-year survival exceeds 85%. These include the development of a highly sensitive and quantitative test that monitors disease burden, a predictive test which examines patients intrinsic sensitivity to tyrosine kinase inhibitor therapy and an assay to determine the functionality of drug transporters, in particular OCT-1 (major active influx pump for Imatinib). Importantly Tim Hughes and Deb White (Adelaide, Australia) stressed that while tyrosine kinase inhibitor (TKI) therapy has improved the outlook for CML patients not all respond well, and that failure to respond is largely related to variability in the intrinsic sensitivity of patients to kinase inhibitors. This can now be assayed for at diagnosis. White demonstrated that understanding the underlying biology of individual patients could in turn provide previously unexplored insight into resistance mechanisms, paving the way for the development of combinational strategies to overcome primary resistance which may in turn reduce development of secondary resistance. Hughes also discussed sustained disease control in some patients who had stopped taking imatinib. His comments may link into an observation made by Jerry Radich (Fred Hutchinson Cancer Research Center, Seattle, USA) that 20% of all individuals have constitutively activated IFNα genes, which might explain why 10-25% of CML patients respond to IFNα treatment and the fact that the response rate to IFNα is determined by the underlying activation status of the immune system. Collectively, these observations suggest a hitherto unexplored role for the immune system in drug-induced disease control and eradication.

**Acute Myeloid Leukemia (AML) - present and future**

Allan Burnett (School of Medicine, Cardiff University, Cardiff, UK) gave an overview of treatment in AML, highlighting the mammoth task of evaluating both currently used treatments, and strategies for evaluating emerging treatments. The use of large randomised trials in combination with cytogenetic and mutation status have seen the improvement of outcome for several groups of young AML patients. However, significant challenges posed are the heterogeneity of AML, the lack of treatments to improve the outcome of elderly patients and the inability of the current trial designs to test new compounds rapidly. Burnett outlined the ‘pick a winner trial design’ that enables the rapid and progressive incorporation of new compounds into the current low dose Ara-C standard of care treatment for elderly patients and allows for agents without promise to be quickly dropped (1).
patient subgroups with poor survival this may transform clinical trial approaches in an era where new treatments are being developed at an increasing rate.

The genomics era of cancer has been led by leukemia with the increasing number of whole genome sequences identifying a plethora of new mutations and initiating new research directions. Thus it was salient for Ross Levine (Memorial Sloan-Kettering Cancer Center, New York, USA) to discuss the profiling of many of these mutations in a large cohort of AML patients (E1900 phase 3 clinical trial) and how outcome classifications have changed. Internal tandem duplications of the FLT3 tyrosine kinase receptor were confirmed as the most frequent alteration (30%) and associated with reduced overall survival, emphasising its continuing position as an important target for therapy. Importantly, integrating mutational analysis with current cytogenetics identified that mutations in \textit{TET2}, \textit{ASXL1}, \textit{MLL-PTD}, \textit{PHF6} and \textit{DNMT3A} could reclassify patients, identified as intermediate risk by cytogenetics alone, as those who will have an adverse outcome (2). Epigenetic modifiers were of particular focus as the mutual exclusivity of \textit{TET2} and \textit{IDH1/2} mutations suggested a shared mechanism of action. This overlapping function was explored further by both Levine and Ari Melnick (Weill Cornell Medical College, New York, USA) who used patient data and animal models to delineate a pathway by which neomorphic mutations in \textit{IDH1/2} proteins lead to increased levels of 2-hydroxy-glutarate in the cell which subsequently inhibit \textit{TET2} function leading to widespread hypermethylation. Melnick also used new techniques of methylation profiling on patient AML patient cohorts to demonstrate that both aberrant hypermethylation (\textit{IDH} mutations) and hypomethylation (\textit{MLL} rearrangements) can be associated with the pathological state, also demonstrating that the MLL-AF9 fusion protein directly leads to a hypomethylated state. He also hinted at studies in progress looking at modules of hypermethylated genes that may lead to chemoresistance in models of leukemia as well as the changing epigenetic landscape in relapsed leukemia. Undoubtedly these findings will lead the way in identifying new treatment strategies for AML.

\textbf{Acute Lymphoblastic leukaemic (ALL) in the genomics era-not lost in translation.}

Charles Mullighan (St Jude Children’s Research Hospital, Memphis, USA) dissected the genetics of early T-cell precursor acute lymphoblastic leukemia (ETP ALL) and discussed methods that have identified mutations that fall into functional clusters; such as activating mutations affecting cytokine receptor and RAS signalling (\textit{NRAS}, \textit{K Ras}, \textit{JAK1/3}, \textit{IL7R}), mutations disrupting normal haematological
development (GATA3, RUNX1, ETV6, IKZF1) and mutations in histone modifying genes (EZH2, SUZ12, EED, SETD2) with novel recurrent mutations found in DNM2, ECT2L and RELN (3). Importantly, many of these genes are also mutation targets in myeloid malignancy, and transcriptional profiling highlighted a similarity between ETP ALL and high risk AML as well as normal HSC. Such detailed profiling has defined ETP as a ‘stem cell leukemia’ and emphasises the importance of this information in diagnosis, classification and downstream treatment approaches. Kathryn Roberts (St Jude Children’s Research Hospital, Memphis, USA) extended this approach in Ph-like high risk pre B-ALL in children to identify activating mutations in the IL-7 receptor, as well as fusion proteins involving ABL1, PDGRFB and JAK. Roberts further demonstrated the therapeutic effectiveness of several small molecule inhibitors using in vitro and in vivo murine models of these novel fusions.

This theme of better drug selection and better patient outcome based on understanding the underlying genetics of the disease was extended by Ross Dickins (Walter & Eliza Hall Institute, Melbourne, Australia). Building on NDLR2008 where Charles Mullighan described the essential role of Ikaros in pre-B cell development and the very poor outcome of patients with these deletions, Dickins and his team sought to understand why Ikaros deletions predict for treatment failure. Glucocorticoids are first-line treatments for ALL patients, with glucocorticoid resistance being linked to poor outcome. Ikaros restoration is associated with restored glucocorticoid sensitivity resulting in apoptosis-driven cell death. This elegant study demonstrates how understanding drug and cellular metabolism pathways are affected by genetic lesions leads to rational drug choices for patients and improved outcome.

**Novel small molecule inhibitors – more new kids on the block!**

Understanding the genetic defects that drive leukemogenesis has facilitated the development of novel small molecule inhibitors. Several speakers used this rationale to develop novel small molecule inhibitors that are currently in pre-clinical and early phase clinical trial. Some of these new approaches include 1) BEZ235, a dual DDR kinase/mTOR inhibitor which has been shown to induce apoptosis in a p53-independent manner leading to enhanced BMF expression, and clearance of the leukaemic burden in animal models (Jake Shortt, Peter MacCallum Cancer Centre, Melbourne, Australia), 2) Blocking the interaction between MYB and p300 to shut down the transformation process whilst leaving normal cells intact (Thomas Gonda, Diamantina Institute, University of
Queensland, Brisbane, Australia), 3) CX-5461 inhibition of Pol 1 transcription; sensitivity to this agent is dependent on wild-type p53 expression, which is maintained in the absence of Arf. Due to changes in nucleolar morphology as a consequence of the inhibition of ribosomal RNA gene transcription, cells die via p53 induced apoptosis and caspase-3 is activated within 2 hours post treatment subsequent to increased p53 and p21 protein levels. Treatment with CX-5461 leads to increased survival in the Eu-Myc lymphoma and the MLL-ENL leukaemic model (Megan Bywater, Peter MacCallum Cancer Centre, Melbourne, Australia), 4) A novel dual-inhibitor of FLT3 and Aurora kinase (CCT241736) has been validated an in vitro model of FLT3 resistance. Oral administration of this drug in preclinical animal models show that CCT241736 overcomes the resistance to the selective FLT3 inhibitor, AC220, caused by secondary FLT3 mutations (Andrew Moore, Institute of Cancer Research, London, UK) 5) inhibition of FLT3 signalling, using the FLT3 inhibitor AC220. Wally Langdon (University of Western Australia, Perth, Australia) has previously shown that a mouse MPN/AML model expressing a RING finger mutant of c-CBL (C379A), which abolishes E3 ubiquitin ligase activity, is dependent on intact FLT3 signalling for disease progression (4). He demonstrated that disease symptoms were reversed with reduced numbers of FLT3+LSK cells, and decreased splenomegaly and extramedullary invasion of myeloid cells. Inhibitor treatment was also associated with reduced proliferation of cells without causing apoptosis, and was itself reversible upon withdrawal of drug with rapid recovery of FLT3+ proliferative cells. These observations are similar to that seen with TKI treatment in CML patients where long term Imatinib provides disease control but not cure. Cure is likely to require combination strategies to destroy the leukaemic stem cells (LSC).

**Cure: a balance between compliance and quality of life? Is there a role for the immune system?**

Catriona Jamieson (Moores UC San Diego Cancer Center, San Diego, USA) spoke about novel therapies to treat myeloproliferative neoplasms (MPN), developed as a result of increased understanding of normal hematopoiesis. MPN granulo-myelo-progenitors (GMP) acquire β-catenin and undergo reprogramming to acquire stem cell characteristics. Polycythemia Vera hematopoietic stem cells (HSC) have the JAK2V617F mutation and the selective JAK2/FLT3 inhibitor, TG101348, decreases mutant burden and leucocytosis, reducing the level of myelofibrosis after 12 months of treatment, but also causes anaemia. The oral Sonic Hedgehog inhibitor, PF04449913, has been successfully used to treat AML in the elderly, reducing marrow blasts and decreasing fibrosis. While these new inhibitors are effective they require prolonged exposure to revert symptoms associated
with MPN. If these therapies are to be effective, particularly in diseases of the elderly, they need to be faster acting and not compromise quality of life.

Only one presentation at NDLR 2012 addressed the role of the immune system in the treatment of leukemia. David Ritchie (Peter MacCallum Cancer Centre, Melbourne, Australia) discussed the potency of natural killer (NK) cells which kill leukemic cells via activating receptors and the release of cytotoxic granules that induce FasL/TRAIL and IFN-γ-mediated apoptosis. Ritchie discussed immune editing of tumours demonstrating that normal myeloid cells have NK ligands, and through analysis of paired diagnosis and relapse samples, can demonstrate further that NK-sensitive AML cells at diagnosis are absent in relapse.

**Myelodysplasia - common but misunderstood?**

Myelodysplasia (MDS), although predisposing to AML and sharing many common genetic lesions, is a very different entity to AML at the cellular level, with apoptosis a prominent feature in early stage disease. Again the theme of identification and understanding the genetic drivers of disease to develop models and new therapies was discussed. Hamish Scott (Centre for Cancer Biology, Adelaide, Australia) demonstrated the power of familial genetics describing GATA2 as a target of mutation in familial MDS/AML (5). Louise Purton (St Vincent’s Institute, Melbourne, Australia), generated a novel mouse model of MDS based on deregulated alternative splicing of the Hoxa1 gene. She is using this model to further understand how MDS occurs, and to identify better treatments for this disease. David Curtis (Australian Centre for Blood Diseases, Melbourne, Australia) used the NHD13 transgenic mouse which is characterised by the expression of the NUP98-HOXD13 to investigate mechanisms and implications of apoptosis in MDS. NHD13 mice develop MDS, AML and ALL and the disease is characterised by prominent apoptosis in myeloid progenitors and pre-malignant progenitors have increased cycling and double strand breaks. Apoptosis in the NHD13 mouse model does not require TNF or FASL and premalignant progenitors do not have increased caspase 8. Early MDS progenitors have reduced BCL2 expression. Expression of BCL2 blocks apoptosis of premalignant progenitors and rescues normal progenitor growth. Blocking apoptosis rescues blood counts and dysplasia but the mice retain a low platelet count. Apoptosis promotes proliferation of pre-malignant cells in MDS, potentially through secretion of factors such as Wnts or prostaglandins. Blocking apoptosis prevents
AML transformation by restoring quiescence and reducing DNA damage, suggesting that blocking apoptosis in MDS may lead to disease control.

**HSC & LSC in the niche – time to leave home**

LSC as the presumptive source of relapse in leukaemia, are masters of ‘laying low’ by maintaining quiescence and localising to sanctuary sites in the marrow, thereby evading therapy. The stromal cells of the marrow play an important role in facilitating this therapy resistance and therefore dissecting out the stem cell-stromal interaction in normal and neoplastic hematopoiesis is essential to target LSC as recently illustrated by the observation that disruption of the CXCR4/CXCL12 axis with CXCR4 antagonists sensitise LSC to conventional chemotherapy. Following and extending this logic, the application of a CXCR4 antagonist AMD3100 to mobilise HSC was examined by Linda Bendall (University of Sydney, Sydney, Australia) who described how the bioactive lipid, S1P, forms a gradient between tissue and blood directing lymphocyte trafficking. Using both pharmacological inhibition of S1P signalling and conditional mouse knockouts of the S1P receptor S1P1 and sphingosine kinase, Bendall and colleagues have shown that S1P is required for HSC egress from the marrow after mobilisation with CXCR4 antagonists (6). As predicted by this model, the use of S1P agonist SEW2871 enhanced HSC mobilisation. The dual use of CXCR4 antagonists with S1P agonists may therefore be an efficient alternative to the use of G-CSF for stem cell mobilisation.

Jean-Pierre Levesque (Mater Medical Research Institute, Brisbane, Australia) discussed the microenvironment of the normal and leukemic marrow. He showed an association between the degree of perfusion of and hypoxia in the bone marrow, with HSC capable of serial repopulation located at the poorly perfused endosteum. Pharmacologic stabilization of the oxygen-labile transcription factor HIF-1α increases HSC quiescence further demonstrating that hypoxia is a quiescence signal for HSC via HIF-1-dependent mechanisms. Leukaemic hyperproliferation alters the marrow environment by increasing hypoxia and stabilizing HIF-1α protein. Leukemic cells have altered hypoxia signalling enabling them to overcome the hypoxia constraint of the leukemic marrow and proliferate in a very hypoxic environment. This results in a stem cell niche hijack by leukemic cells. These observations are in support of the use of hypoxia-induced pro-drugs to target LSC.

**Conclusions**
Jerry Radich paraphrased Confucius saying that “real knowledge is to know the extent of one’s ignorance” and it is evident that an integrated understanding of basic cellular, molecular and metabolic pathways are required to develop effective new therapies that sustain clinical responses. Many new findings were presented at the NDLR2012 meeting and it is clear that considerable recent progress has been made from genomics and epigenetics that have potential to improve patient classification, treatment selection and outcome. Future challenges require an understanding of the role of the immune system in small molecule inhibitor-mediated disease control.
References

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