

NCI White Paper: Think Tank on Molecular Targets in Lymphoid Malignancies

The meeting of the *Think Tank on Molecular Targets in Lymphoid Malignancies* held at the NIH on August 24-26, 2005, brought together experts in the fields of leukemia, lymphoma, and multiple myeloma to review current targeted and non-targeted cancer therapies and to identify critical molecular pathways that might serve as new targets for cancer therapy. This report summarizes the findings of a breakout session and the presentations made during the course of the meeting.

Breakout Session: Opportunities and Needs

There was collective agreement among discussion participants of the necessity to integrate correlative studies into all clinical trials in lymphoma; molecular classification of the tumor through a complete genomic analysis should become a routine procedure. Recent advances in microarray and gene chip technology have allowed elucidation of the significant molecular heterogeneity of these diseases, while recognition of the involvement of microRNAs (miRNAs) has altered our understanding of both cell and cancer biology. Further research is needed, however, to identify the critical gene pathways for tumor proliferation, survival, and apoptosis either common to all or specific for each lymphoid malignancy, and then to apply these findings to other cancers—it is however premature to prioritize molecular targets as there is still too much to be learned about the biology and pathology of lymphoid malignancies.

The ability to classify tumors according to aberrant genetics, patient prognosis, and response to therapy is of significant benefit because it enables each patient to be offered the optimum treatment regimen and allows stratification of subjects in clinical trials. During these trials, patient samples should be collected and appropriately stored before, during, and after treatment to assess effects on the targeted moiety. Trials should measure real-time pharmacokinetic and pharmacodynamic parameters to determine drug distribution and concentration relative to toxicity and clinical response. Validated molecular surrogates are also necessary as a measurement of clinical outcome. Traditional clinical study designs may need to be rethought in order to combine agents based on an understanding of disease-complementary targets rather than on toxicity. Phase 0 trials should also be considered, although their associated ethical issues would need to be addressed first.

Innovative microarray, imaging, NMR, and bioinformatics technologies are being employed to identify genetic abnormalities and develop new therapeutic agents. Better preclinical models can help to evaluate these new agents by measuring synergistic anti-tumor efficacy as well as direct effects on drug targets. Recognizing the importance of the microenvironment in myeloma pathogenesis, a new human bone marrow xenograft model is currently being developed. New agents, however, need to be evaluated in the clinical setting, and patients are a valuable but limited resource. Cooperative multicenter trials will be the only mechanism to accrue the required number of patients to detect statistically significant results in Phase II/III studies. The challenge of selecting which promising agents to test in these studies will be made easier with comprehensive correlative data collected from earlier-stage clinical trials.

Opportunities for Leukemia (ALL, CLL): One priority is to understand why the prognosis for adult patients with acute lymphocytic leukemia (ALL) is so much worse than in pediatric ALL; the disparity may be related to the increased incidence of the Philadelphia chromosome (Ph1)—and corresponding involvement of the *Bcr-Abl* fusion gene—in older patients with ALL. Drugs that inhibit Bcr-Abl tyrosine kinase (e.g., Imatinib) are promising, but there are only a few targeted agents to treat ALL. Conducting more aggressive clinical trials in adult patients with ALL is one favored option, as is the evaluation of therapy with pediatric chemotherapy regimens, and drugs effective in patients with chronic lymphocytic leukemia (CLL) (e.g., Alemtuzumab). In addition, several of the cell-surface antigens specific to CLL, including some involved in cell-microenvironment interaction, may be potential therapeutic targets in this disease. Bcr- and NF- κ B signaling components, as well as the TNF family ligands BAFF and APRIL, are also considered promising targets for therapy. However, the number of promising new agents in development to target critical pathways such as the NOTCH signaling pathway, mammalian target of rapamycin (mTOR), and NF- κ B, exceeds our capacity to conduct Phase II/III trials.

Opportunities for Lymphoma (DLBCL, MCL, FL): Subclassification of diffuse large B-cell lymphomas (DLBCL) has revealed several subtype-specific targets for therapy. NF- κ B target genes are upregulated in activated B-cell-like (ABC) and primary mediastinal B-cell lymphoma (PMBL), but not in germinal center B-cell-like (GCB) subtypes. I κ B kinase (IKK) inhibitors are in development and may be effective against ABC DLBCL and PMBL as well as Hodgkin's and MALT lymphomas. Rituximab is showing clinical efficacy as a single agent, and an understanding of its downstream targets will be essential for designing combination studies. Genetic aberrations in cell cycling and DNA damage response are considered highly promising targets for therapy: cyclin D1 dysregulation, amplification of CDK-4, deletions of the CDK inhibitor p16(INK4a), and overexpression of BMI-1 are common features of MCL and have relevance to other cancers. Similarly, thalidomide and its immunomodulatory derivative lenalidomide are proving effective as single and combination agents in different lymphoid malignancies.

Opportunities for Myeloma (MM, MGUS): The prognosis of patients with Ig amyloid deposition is poor, prompting a need for agents that block Ig secretion, possibly by targeting X-box binding protein-1 (XBP-1) or IRE1 kinase. Also considered of importance are efforts to determine the antigen promoting clonal expansion in Ig amyloidosis. For multiple myeloma itself, novel single and combination agents that target tumor cells and the bone marrow microenvironment hold promise against tumor-cell growth, survival, and drug resistance. These agents can be evaluated *in vivo* in xenograft models of engrafted bone marrow cells. Although nearly all multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) tumors have dysregulated and/or increased cyclin D expression, more research is needed into the molecular differences that govern progression from MGUS to MM. Potential targets for multiple myeloma associated with cell survival and proliferation, cytokine signaling, and angiogenesis are extensive—the proteasome and aggresome, D-type cyclins, CKS1B, FGFR3, STAT3, IKK, PI₃K, IGF1, and VEGF, among others. The involvement of the

Wnt signaling pathway and its antagonist Dkk1 in osteoblast differentiation and osteolytic bone lesion development is of critical importance given the morbidity and mortality of bone disease in myeloma.

Presentations

Think Tank keynote speaker **Dr. Suzanne Cory** discussed human B-cell lymphomas in light of recent developments in the study of chromosomal translocations, *myc*, and Bcl-2 family proteins. Her focus was on Bcl-2 proteins as new targets for cancer therapy, particularly ABT-737, a small-molecule antagonist of Bcl-X(L) and Bcl-2 that has demonstrated efficacy as a single agent by inducing apoptosis in lymphoma and small-cell lung carcinoma lines as well as in primary patient-derived FLL and CLL cells.

Basic Science Session

Dr. Frederick Alt explained that in mice lacking the double strand break (DSB) repair factors H2AX, 53BP1, and MDC-1, AID-induced switch-region DSBs are not held together for repair during class switch recombination (CSR), resulting in large numbers of broken ends within the IgH locus and increased genomic instability. An inverse correlation between the frequency of IgH locus breaks and reduced CSR efficiency in MDC-1-, H2AX-, and 53BP1-deficient B cells (mild, moderate, and severe, respectively), suggests a specific order of factor activity during DSB repair. **To view Dr. Alt's PowerPoint presentation, [click here](#).**

Dr. Klaus Rajewsky discussed his recent results of studies investigating the regulation of the B-cell antigen receptor (BCR), and its role in normal and malignant B-cell development and survival. He also described the application of Cre/loxP conditional gene targeting to modeling human diseases in mice, with an emphasis on generating models of human mature B-cell lymphomas by deregulating NF- κ B signaling pathways.

Dr. Tak Mak described several cancer-associated cell survival genes that are potential targets for novel therapeutic agents and presented mouse models developed to characterize apoptotic and cell survival pathways. Mice expressing a mutant form of cytochrome c (KA allele) retained normal mitochondrial electron transport function but failed to activate Apaf-1. Differential susceptibility to gamma-irradiation induced apoptosis in KA/KA and Apaf-/- thymocytes suggests a cytochrome c independent caspase activation pathway in these cells. **To view Dr. Mak's PowerPoint presentation, [click here](#).**

Dr. Chi Dang described how analysis of genes regulated by c-Myc reveals an over-representation of those genes involved in mitochondrial biogenesis. Myc appears to play a key role in a switch to glycolytic metabolism during cell proliferation or tumorigenesis. Target genes can be divided into those that respond "physiologically" vs. those that respond "pathologically" to either absent, high, or low levels of conditionally induced *c-myc in vitro*. **To view Dr. Dang's PowerPoint presentation, [click here](#).**

Novel Strategies for Drug Development Session

Dr. Ari Melnick explained that BTB domain peptide inhibitors may constitute a novel therapeutic approach against B-cell lymphomas. A peptide has been developed that specifically binds Bcl-6, blocking corepressor recruitment and the establishment of silenced chromatin, reactivating natural Bcl-6 target genes. Peptide blockade in Bcl-6-positive lymphoma cells caused apoptosis and cell cycle arrest, and *in vivo* arrested lymphoma growth without detectable toxicity.

Dr. Maurizio Pellecchia discussed the use of structure-based design aided by techniques such as NMR spectroscopy and molecular modeling to design, synthesize, and characterize novel classes of small molecule leads capable of antagonizing the anti-apoptotic effects of Bcl-2 and Bcl-X(L). These small molecules can be used as pharmacological tools for mechanistic studies and target validation, and hold great potential for translation into novel therapeutic drugs.

Lymphoid Leukemia Session

Dr. Thomas Kipps' presentation emphasized that an analysis of genomic aberrations in patients with chronic lymphocytic leukemia (CLL) is critical for predicting disease progression and survival, and can also inform the design of risk-adapted treatment strategies. The initial resistance to CD95-mediated apoptosis caused by CD154 gene therapy in patients with CLL may be caused by high-level expression of X-linked inhibitor of apoptosis protein (XIAP); efforts are underway to develop specific XIAP inhibitors as therapeutic agents.

Dr. A. Thomas Look reported that disruption of the NOTCH signaling pathway may be required as a first step in the induction of human T-cell acute lymphoblastic leukemia (T-ALL), regardless of the additional genes that ultimately become mutated. Agents that target the NOTCH pathway are being developed as mutationally activated forms of NOTCH1 are still dependent on enzymatic cleavage for activity. A clinical trial of one such drug, a gamma secretase inhibitor, has recently been opened at the Dana-Farber Cancer Institute to specifically target the NOTCH pathway in children and adults with relapsed or refractory T-ALL.

Dr. Carlo Croce reported the identification of two miRNA genes, *miR-15* and *miR-16*, that are deleted or downregulated in 68% of patients with CLL. These genes are ubiquitously expressed in normal human tissues. A unique 13-gene miRNA expression signature can, in conjunction with the level of ZAP-70 expression and the presence or absence of *IgVH* mutations, differentiate two forms of CLL with different disease prognoses. Bcl-2 has recently been identified as a target of both *miR-15* and *miR-16*, and antisense *miR-15* and *miR-16* will downregulate Bcl-2 expression and cause apoptosis in certain leukemic cell lines. **To view Dr. Croce's PowerPoint presentation, [click here.](#)**

Lymphoma Session

Dr. Wyndham Wilson emphasized that an understanding of lymphoma subtypes is critical when designing and conducting clinical trials of new therapeutic regimens. Clinical trials of targeted therapy include: EPOCH chemotherapy with bortezomib in ABC DLBCL tumors; the CDK inhibitor flavopiridol, mTOR inhibitor Temsirolimus, and thalidomide and lenalidomide in MCL; and the HDAC inhibitor SAHA in DLBCL. Optimizing the chemotherapy administration schedule in the dose-adjusted EPOCH regimen has significantly increased the 5-year survival rates in patients with Bcl-2-positive ABC- and GCB-type DLBCL tumors. **To view Dr. Wilson's PowerPoint presentation, [click here](#).**

Dr. Louis Staudt discussed separating lymphoid malignancies into molecularly and clinically distinct subgroups by gene expression profiling to identify and target signaling pathways necessary for abnormal proliferation and survival. New molecular targets are being identified in “Achilles heel” screens for small hairpin RNAs (shRNAs) that block the proliferation or survival of cancer cells. Development of an inducible shRNA retroviral expression library targeting 2,500 human genes enabled identification and verification of shRNAs that target genes regulating the cell cycle, transcription, and splicing. Several of these shRNAs were only toxic to particular subtypes of lymphoma or myelomas and define a new class of therapeutic targets in lymphoid malignancies. **To view Dr. Staudt's PowerPoint presentation, [click here](#).**

Dr. Laura Pasqualucci presented her recent work related to the elucidation of mechanisms of genetic lesions in lymphoma, the recent finding that Bcl-6 can suppress p53-dependent and -independent growth arrest and apoptosis in GCB cells, and the potential of Bcl-6 as a therapeutic target.

Dr. Margaret Shipp explained that the significant clinical and genetic heterogeneity of DLBCL requires novel targeted therapeutic agents directed to each disease subtype. A comparison of the NF- κ B target gene signatures of primary mediastinal large B-cell lymphoma (MLBCL) and DLBCL subtypes identified differences that may indicate alternative NF- κ B activation mechanisms in these tumor cell types.

Brief Talk (*Selected from submitted abstracts*)

Dr. Julie Teruya-Feldstein introduced the ARF-specific transcriptional repressor Pokemon (POK erythroid myeloid ontogenic factor) as a possible agent for targeted lymphoma therapy. Mice overexpressing Pokemon in immature B- and T-lineage cells develop aggressive, rapidly fatal lymphomas, while loss of Pokemon *in vitro* results in aberrant ARF upregulation, and unresponsiveness to oncogenic stimuli. **To view Dr. Teruya-Feldstein's PowerPoint presentation, [click here](#).**

Multiple Myeloma Session

Dr. Kenneth Anderson discussed the use of oncogenomics to inform drug development and protocol design to target the tumor cell in its microenvironment. His lab developed an *in vivo* model in which IL-6-dependent human MM INA-6 cells are engrafted into human fetal bone chips implanted in SCID mice. This model is being used to evaluate drugs that target human MM cells within the BM microenvironment. **To view Dr. Anderson's PowerPoint presentation, [click here](#).**

Dr. Michael Kuehl reported that analysis of chromosome content indicates that there may be two pathways involved in the tumor pathogenesis of MGUS and MM —non-hyperdiploid with one of five diverse but recurrent IgH translocations, or hyperdiploid with multiple trisomies involving eight different chromosomes. Patterns of cyclin D expression and translocation allow classification of MM into eight groups with differing gene expression patterns, biological and clinical features, response to therapy, and prognosis. For example the hyperdiploid “D1” group is virtually absent in extramedullary MM tumor cells and cell lines, suggesting a particularly strong dependence on interaction with the bone marrow microenvironment and a possible therapeutic target. **To view Dr. Kuehl's PowerPoint presentation, [click here](#).**

Dr. John Shaughnessy presented a 70-gene signature in patients with MM that can define 3 groups with different disease prognoses. Overexpression of *CKS1B* correlates with poor disease outcome in patients with MM. *CKS1B* mRNA and protein levels both inversely correlate with nuclear p27^{Kip1}, while targeting *CKS1B* with siRNA induces upregulation of p27^{Kip1} expression as well as apoptosis in myeloma cell lines. **To view Dr. Shaughnessy's PowerPoint presentation, [click here](#).**

Dr. Leif Bergsagel discussed the molecular classification of MM and described transgenic mouse models designed to investigate the timing of malignant transformation in B cells as a determinant of tumor phenotype. The usefulness of these models for preclinical therapeutic and prevention studies was also highlighted.