Cancer Research Foundation
Integrated Leukemia Project at the University of Chicago

2010/2011 Research Highlights

Team 1 – Genetics

Investigators: Onel, Le Beau, White, Lussier, Larson

Goals: 1) Identify genetic risk factors that may contribute to t-AML susceptibility.
2) Develop a genetic profile of t-AML.

Dr. Onel has been identifying single nucleotide polymorphisms (SNPs), single variations in DNA sequences, associated with increased or decreased risk for t-AML. Previous studies indicated that distinct t-AML subtypes are the result of distinct gene-exposure interactions, an observation that may have significant implications for identifying patients at risk for t-AML. To follow up on these results, a much larger genome-wide association study (GWAS) in t-AML is being performed. Although no single variant surpassed the threshold for genome-wide significance, nearly a dozen SNPs of potential interest were identified. Similar to what was observed in the earlier t-AML GWAS, many of the variants identified in this study are in or near genes that are ion channels or are involved in neuronal processes, although it is too early to speculate on the significance of this finding.

Substantial progress has also been made in the analysis of the genetic profile of t-AML. A database was created merging two separate databases containing patient demographics, clinical data, and tissue bank records. Using this new entity, Dr. Le Beau identified and stored leukemia samples from 85 patients. DNA and mRNA were extracted from these samples and genetic analysis is being performed, including high-throughput sequencing of gene transcripts by Drs. White and McNerney to identify functional mutations.

Drs. White and McNerney also continue the next-generation transcriptome sequencing of t-AML patient samples and *de novo*, or primary, AML samples. Initial analysis of the expression profiles of the samples has revealed that even though t-AMLs and *de novo* AMLs have similar cytogenetic abnormalities, they have fundamental differences at the genetic level. The transcripts that are differentially expressed between t-AMLs and *de novo* AML have also been identified. Additionally, seven mutations have been found to date, four of which have a known role in t-AML. The frequency of the remaining three mutations, which have not been previously reported in t-AML, is currently being assessed across a larger panel of patient samples. These findings are being used to identify the molecular networks that are perturbed in t-AML and to develop more effective clinical trials.

Team 2 – Molecular Pathways

Investigators: Cunningham, Singh, Le Beau, White, Lussier

Goal: Identify molecular pathways involved in the production of healthy blood cells.

Drs. Cunningham and Singh are identifying gene regulatory networks that govern normal blood cell production. Through this process, they will be able to identify gene networks that are deregulated in leukemia stem cells (LSCs) and ultimately develop drugs to target these specific defects. They are

currently focusing on two important transcription factors, EZH2 and PU.1, specific proteins that are known to play a role in leukemia.

EZH2 is known to be a regulatory factor in chromatin architecture, which appears to be critical in a subset of patients with t-AML. A lentiviral knockdown construct was developed to further investigate EZH2. This technique, commonly used in genetic studies, reduces the expression of a specific gene in question allowing researchers to study the effects that may result in cells and/or animal models. A similar construct is also being constructed to study PU.1, a transactivator whose loss is critical in leukemic hematopoietic progression.

In addition, Dr. Cunningham is examining the organization of genes and other structures within the nucleus of cells. The assays for chromatin structure and nuclear architecture are being validated so that t-AML stem cells can be tested to determine if they have a unique architecture that contributes to the development of leukemia.

Team 3 – Drug Responses and New Therapies

Investigators: Onel, Dolan, Huang, Gurbuxani, Lussier

Goals: 1) Determine how genetic variations influence an individual's response to drugs.
2) Screen chemical libraries to identify new chemicals that inhibit leukemia growth.

Drs. Dolan and Huang are identifying single nucleotide polymorphisms (SNPs), changes found at a single site in DNA, associated with cellular sensitivity to chemotherapeutic agents (e.g., cytosine arabinoside (AraC), daunorubicin, and etoposide) used to treat AML and t-AML. They are developing a cell-based model for discovery of genotype-phenotype relationships, and plan to take those discoveries to clinical trials with the ultimate goal to identify patients upfront who are likely to have an adverse reaction or non-response.

Researchers have generated chemotherapy induced cytotoxicity on lymphoblastoid cell lines (LCLs) derived from individuals from different human geographical areas. They have also optimized drug-induced cell death, a phenotype that may more closely resemble AraC-induced cell death in tumor cells. Novel methods are being developed to use next-generation datasets to evaluate rare variants that contribute to cellular sensitivity to chemotherapeutic agents used to treat AML. Investigators also plan to utilize subsets of data from the International HapMap effort, the National Institutes of Health (NIH) 1000 Genome Project, and NIH-NIEHS resequencing effort to evaluate SNPs within the candidate and novel genes for their association with sensitivity to AraC as well as other agents used to treat AML (daunorubicin, etoposide). This is highly translational because the ultimate goal is to bring the most informative SNPs into a clinical trial.

In collaboration with Drs. Dolan and Huang, Dr. Onel is conducting studies targeting cell death to discover new therapies for t-AML. Through various testing on nine AML cell lines, the goal will be to identify a death signature that is elicited by a variety of different drugs that cause AML cell death. In this way, core pathways mediating cell death in response to chemotherapeutics will be identified. A signature can then be validated by examining its association with outcome in primary patient AML samples and will then be used to screen compounds for those that elicit cell death through the gene signature. In turn, these compounds will become attractive candidates as new targeted therapies for AML.

Team 4 – Mouse Models

Investigators: Singh, Cunningham, Le Beau, Lussier

Goal: Study t-AML using mouse models.

Drs. Cunningham and Singh have successfully established a mouse model of human t-AML. Specifically, three cohorts of animals have been injected with leukemia cells derived from three unique patient samples. Initial analysis has demonstrated that these animals have engrafted with human leukemia stem cells (LSCs). Secondary experiments will be performed to establish the transplantability and long term repopulating potential of these cells. Once validated, LSCs derived from the model will be genomically profiled and screened for sensitivity to new chemicals that may inhibit the progression of leukemia. Normal hematopoietic stem cells derived from human umbilical cord blood were also transplanted into the mouse strain and repopulation was observed, although at suboptimal levels. This system is currently being optimized with further experimentation.

Dr. Cunningham will work with Dr. Le Beau to develop a human LSC model to examine the function of Egr1, a critical gene in the etiology of t-AML. It is expected that these studies will provide significant insights into the targets of this tumor suppressor in human blood stem cells. Other animal models are being established which will allow researchers to further analyze two important transcription factors identified by Team 2, EZH2 and PU.1. These specific proteins are known to play a role in leukemia. Successful completion of these studies should also reveal novel therapeutic targets for future exploitation, with the ultimate expectation of improvements in the treatment of both AML in the elderly, and therapy-related AML.

Team 5 – Clinical Trials

Investigators: Godley, Odenike, Stock, Larson, Dolan, Huang

Goals: 1) Design and conduct clinical trials of new t-AML drugs to maintain patients in remission.

2) Determine the effect of drugs by collecting patient samples for genetic testing.

A new treatment approach was developed for patients with therapy-related myeloid neoplasms (t-MN), using a combination of chemotherapy (cytarabine (AraC)) and mitoxantrone (a DNA hypomethylating agent), followed by hematopoietic stem cell transplantation (Godley et al., *Leuk Lymphoma* 51: 995-1006, 2010). Many of the 32 patients in this trial had previously received significant amounts of chemotherapy. Despite this, the vast majority of patients were able to tolerate the combined therapy, with an excellent response rate and minimal toxicity. Patient samples from this trial are being genetically tested and analyzed in order to continue to gain a better understanding of the complex networks that trigger therapy-related leukemias, as well as to help identify the cellular pathways responsible for normal blood production.

A retrospective analysis of the regimen published above has also been completed in 78 adults with high risk leukemias to evaluate its efficacy in a broader group of patients. It was concluded that the protocol was an effective and well-tolerated regimen for this patient population. Given these promising results, further feasibility trials are currently being designed to test current hypotheses to produce prolonged disease free-survival. Key patient samples would be obtained throughout any

testing that could be analyzed by other Team investigators to genetically and biologically validate all clinical results obtained.

Dr. Godley has developed a chemical assay for measuring 5-hydroxymethylcytosine, a newly identified base found in DNA. This may help clinicians recognize which patients respond favorably to hypomethylating agents and which are resistant. Moreover, many patients with AML have mutations in their genes that affect the level of this particular base that is present in their DNA. A clinical trial being developed that will take advantage of these findings.

Team 6 – Atlas of Therapies

Investigators: Lussier with all team members

Goals: **1) Use bioinformatical approaches to model the cellular networks that influence a patient's response to therapy.**

2) Develop a simulator, the Atlas of Therapies, which will enable researchers to simulate the effect of new drugs in t-AML patients.

Dr. Lussier has successfully completed the Functional Analysis of Individual Microarray Expression (FAIME) study for personalized therapy of t-AML. To increase the utility and reproducibility of gene signatures, Dr. Lussier has developed an analytical approach, FAIME, to derive "personal functional signatures" in which a patient's gene expression is translated into molecular functions and pathways. These functional signatures allow individual predictions and dramatically reduce the number of patients required for validation as compared to conventional gene expression signature classifiers that generally require a few hundred patients. The validation of FAIME in t-AML and/or related leukemias is being pursued.

Dr. Lussier is also encoding every clinical narrative (pathology, radiology, hospital discharge summaries, etc.) associated with biospecimens, including t-AML. Several interfaces are being completed, including those involving other computer systems (e.g. Cloud space, grid, clinical warehouses) and human displays for rapid querying and synthesizing vast amount of clinical data. This work will accelerate requisitions for additional specimens for systems biology research. In addition, Dr. Lussier is applying protein interaction modeling, in collaboration with Drs. White and McNerney, to interpret mutations measured in t-AML specimens.