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Introduction

University of Chicago researchers are unraveling the complex network of molecular interactions that influence the development of leukemia. Building off the generous support of the Cancer Research Foundation, we have made significant advances in understanding the genetics of therapy-related acute myeloid leukemia (t-AML). Our multi-disciplinary team of scientists and clinicians are gaining a global perspective of the biological process that underlie t-AML using a systems biology approach. Their discoveries are guiding the development of new personalized therapies for leukemia, as well as new strategies to prevent the development of t-AML.

Project 1 – Identifying the Genetic Signature of Therapy-Related Leukemias

Team:  Onel, Le Beau, White, Larson
Goals:  1) Identify genetic risk factors that may contribute to t-AML susceptibility.
       2) Develop a genetic profile of t-AML.

We are using two complementary approaches to genetically profile t-AML. First, Drs. Onel and Larson are examining DNA from healthy (non-malignant) tissues to identify genetic susceptibilities to t-AML. Their findings will lead to the discovery of new biomarkers that gauge disease risk, and will also guide therapy at the time of initial cancer diagnosis to minimize the subsequent risk of t-AML. Second, Drs. Le Beau and White are mapping all of the cooperating lesions responsible for the development of t-AML by profiling the leukemia cells of the same patients. These complex datasets are being analyzed by computational methods to identify key cellular processes that are deregulated in t-AML.

Genetic Susceptibility and It’s Contribution towards t-AML Development

Although genome wide association studies (GWAS) have led to the discovery of numerous variants indisputably associated with a number of complex traits, the clinical utility of these discoveries has been limited. The research team hypothesized that one reason for this limitation is that it is virtually impossible to account for inter-individual variability in environmental exposures contributing to these traits in these studies. Because different
exposures elicit different cellular response pathways, each with its own set of genetic determinants, they reasoned that the attenuation of genetic effects was the inevitable consequence of not accounting for differences in “environment” among individuals in genetic studies.

To overcome this limitation, Dr. Onel’s laboratory performed a GWAS of radiation-therapy (RT)-induced second malignant neoplasms (SMNs) in survivors of Hodgkin lymphoma (HL). Here, the etiological exposure, RT, was common to both cases and controls; therefore, the primary distinguishing factor between these groups was their genetic predispositions towards SMNs. Using a discovery set of only 96 cases and 82 controls, they identified and functionally confirmed a highly penetrant locus associated with RT-induced SMNs following HL. They then performed a similar GWAS to investigate predispositions towards t-AML, with 228 cases and almost 1000 controls, followed by an analysis of the 100 variants most associated with t-AML in another 200 cases and 400 controls. Here, they were unable to identify any variants surpassing the threshold for genome-wide significance, even when they subdivided the patients by t-AML subtype. Dr. Onel’s laboratory also performed a GWAS on de novo AML with about 1000 cases and 2600 controls; again, no variant achieved genome wide significance.

Intriguingly, Ley and colleagues recently reported that the clonal diversity of hematopoietic stem cells (HSCs) in healthy cancer-free individuals was directly correlated with age. This suggests that as the result of the accumulation of mutations over the course of time, multiple stem cell clones develop within each individual as part of the natural aging process. This, then, provides more opportunities for stochastic (or chemotherapy-induced) mutational events leading to cancer to occur. This could explain why the incidence of both AML and t-AML increase with age.

A prediction that emerges from these data is that if both de novo AML and t-AML result from the chance acquisition of leukemogenic mutations by one of a number of clones existing in an individual, then both diseases should have low heritability. Indeed, when Dr. Onel’s laboratory
performed heritability analyses for de novo AML and t-AML, they found that the heritability for both diseases was virtually nonexistent. This suggests that common inherited variants contribute very little to risk for either disease, which agrees with the results from Dr. Onel’s GWAS studies, and implies that they are largely caused by chance or environmental events.

Although Dr. Onel’s data indicate that common variants contribute little to AML susceptibility in the general population, it is well known that AML is a component of a number of familial cancer predisposition syndromes caused by very rare, but high penetrance, mutations. One hallmark of these genetic cancer syndromes is the development of multiple cancers in a single individual. The research team hypothesized that a subset of patients phenocopied (mimicked) t-AML, but in reality had unsuspected cancer predisposing conditions, such as Li-Fraumeni syndrome (LFS). Indeed, of the 228 t-AML cases evaluated, 20% have evidence suggestive of an underlying cancer predisposition (3+ primary cancers; breast cancer under the age of 50; antecedent sarcoma, etc). Of note, the researchers do not have family history for these cases. Therefore, this 20% must be regarded as a conservative estimate. Supporting the hypothesis that a subset of t-AML patients actually do not have t-AML, but instead have an underlying syndrome is a paper by Tim Ley and colleagues, in which they reported that the first putative t-AML patient whose germline genome was sequenced actually had a known deleterious mutation in TP53 and, therefore, had LFS. To test this hypothesis, Dr. Onel’s laboratory is currently sequencing the exomes of 27 t-AML patients with a personal cancer history suggestive of a cancer predisposition syndrome.

These syndromes are associated with cancer susceptibility not because the gene mutations underlying them cause cancer; rather they are permissive for further mutation acquisition by, for example, inactivating DNA repair pathways or apoptotic pathways that delete cells with potentially oncogenic somatic mutations. Thus, as a result of these permissive mutations, individuals with these syndromes are prone to develop increased numbers of stem cell clones relative to normal individuals of the same age, thereby providing many more opportunities for one clone or another to undergo transformation. The team surmises that this permissiveness
for subsequent stochastic mutational events is the basis for cancer predisposition in affected individuals.

Thus, Dr. Onel’s data have the potential to revolutionize the way we think about AML and t-AML risk, and provide a new evolutionary model for these diseases. If the etiology of both diseases in the general population is largely due to environmentally/chemotherapeutically induced or stochastic mutations driving clonal selection, then it should be possible to detect the pre-leukemic HSC clone in individuals before it develops into AML. If so, its mutational spectrum can be compared to each individual’s normal genome to determine what made that clone a “set-up” for transformation. In addition, its mutational spectrum can be contrasted against that of the leukemic clone to determine what additional mutations were required for transformation. The consequences of these “high risk” clones can then be modeled in animals. This might someday facilitate prescreening of patients receiving chemotherapy to identify those with “high risk” clones, and may permit therapies targeting these clones to destroy them, thereby eliminating risk for t-AML.

**A Global Profile of Genetic Mutations Acquired by t-AML**

Dr. Le Beau has made substantial progress in defining the full complement of cooperating lesions responsible for the development of t-AML by profiling the *leukemia cells of the patients* studied by Dr. Onel. To identify somatically-acquired genomic changes, i.e., causative mutations acquired by the leukemia cells, she analyzed 76 t-AML patients using a high-resolution copy number analysis with the Affymetrix Genome-Wide Human SNP Array 6.0 (resolution of 5kb) also used above by Dr. Onel. Forty-five patients had abnormalities of chromosomes 5 and/or 7, 21 had recurring translocations or other abnormalities, and 10 had a normal chromosomal complement in their malignant cells. This analysis was highly informative, and revealed the following results:
- t-AMLs have an average of 7.9 copy number alterations (gain or loss of genetic material) with a range of 0-62 alterations per case. This contrasts with AML de novo, which typically has <1 copy numbers alterations per case.
- The loss of chromosomal material, implicating the involvement of tumor suppressor genes, far outweighs the gain or amplification of genetic material, which would implicate oncogenes (ratio 1.7:1).
- Leukemias with a del(5q) show genetic instability with an average of 9.7 copy number alterations vs. 2.3 in leukemias with -7/del(7q) without 5q abnormalities, or 2.1 in leukemias with recurring translocations.
- Four patients had small focal deletions that were submicroscopic, i.e., not visible by conventional cytogenetic analysis of chromosomes through the light microscope. The deletions encompassed the interval containing four genes, CUX1/POLR2J/ORAI2/PRKRP1, which all had down-regulated expression, making them candidates for a myeloid leukemia suppressor gene. The identification of CUX1 in this interval confirms the findings of Dr. McNerney and White described below.

To complement the above analysis, Dr. Le Beau’s laboratory has initiated high-throughput genomic sequencing of 35 of these cases, using whole exome sequencing (which provides partial sequence analysis of the genome by focusing on the protein coding segments of the genome). On average, they detected 22.2 mutations per case: 12.5 mutations in leukemias with abnormalities of both chromosomes 5 and 7; 42.7 in del(5q) leukemias; 23.3 mutations in -7/del(7q) leukemias, and 17.6 mutations in leukemias with a normal karyotype. Dr. Le Beau’s laboratory is also using the mutational profile of t-AML to identify the key cellular processes that are deregulated in t-AML, and to identify cellular pathways that cooperate in this process. For example, the analysis described above revealed that del(5q) occurs in conjunction with mutations of the TP53 gene. The TP53 protein regulates the cellular response to stress and DNA damage; deregulation of this pathway leads to genomic instability and the accumulation of additional mutations.
Deficient Levels of CUX1 Contribute to Leukemia Development

Over the past year, Drs. White and McNerney have also made significant progress in developing a genetic profile for t-AML. Loss of chromosome 7 and del(7q) [-7/del(7q)] are recurring cytogenetic abnormalities in hematologic malignancies, including AML arising de novo and therapy-related myeloid neoplasms. These genetic abnormalities occur in 9% of de novo AML and 50% of t-AML, and are associated with an adverse prognosis. Despite intensive effort by many laboratories, the putative myeloid tumor suppressor(s) on chromosome 7 has not yet been identified.

Dr. White’s laboratory performed transcriptome sequencing (sequencing of the expressed mRNAs of active genes) and SNP array analysis on 35 patient samples of de novo and therapy-related myeloid neoplasms, half with -7/del(7q). They identified a 2.17 Mb commonly deleted segment on chromosome band 7q22.1 containing CUX1, a gene encoding a homeodomain-containing transcription factor. In one case, CUX1 was disrupted by a translocation resulting in a loss-of-function RNA fusion transcript. CUX1 was the most significantly differentially expressed gene within the commonly deleted segment and was expressed at haploinsufficient levels (a reduction of ~50%) in -7/del(7q) leukemias. Haploinsufficiency (expression of a single copy of a gene while the other copy is inactivated by loss or mutation) of the highly conserved ortholog, cut, led to hemocyte overgrowth and tumor formation in Drosophila melanogaster (fruitflies) [see Figure 1].

Made possible by the close interactions shared among research teams, they quickly validated these results in mice by collaborating with Dr. Cunningham’s group (see Project 4). They observed that haploinsufficiency of CUX1 gave human hematopoietic cells a significant engraftment advantage upon transplantation into immunodeficient mice. By evaluating RNA-sequencing data, Dr. White’s group also identified a CUX1-associated cell cycle transcriptional gene signature, suggesting that CUX1 exerts tumor suppressor activity by regulating proliferative genes. These data identify CUX1 as a conserved, haploinsufficient tumor
suppressor frequently deleted in myeloid neoplasms. These results were recently published in the journal *Blood* (McNerney et al., 2013).

Figure 1. Haploinsufficiency of the *CUX1* homologue, *cut*, leads to hematopoietic overgrowth and formation of melanotic pseudo-tumors in *Drosophila melanogaster*. Larvae (right) and pupae (left) develop abnormal black masses (outlined by red squares) upon *cut* knockdown. Punctate GFP+ hemocytes can be appreciated in the larval stage. *CUX1* is frequently deleted in high-risk acute myeloid leukemia and haploinsufficient levels lead to increased growth of human hematopoietic cells, demonstrating the conservation of *CUX1/cut* from invertebrates to humans.

The White and McNerney laboratories are currently identifying the regulatory network of the *CUX1* transcription factor. This objective will be achieved by testing the working hypothesis that *CUX1* regulates and interacts with critical myeloid transcription factors to control myelopoiesis and growth. This hypothesis will be tested using chromatin immunoprecipitation of DNA gene regulatory regions bound by *CUX1*, followed by sequencing (ChIP-seq) in human myeloid leukemia cells and overlaying this binding site information with the rich and extensive datasets provided by ENCODE (Encyclopedia of DNA Elements). The rationale for these experiments is that successful completion will identify the genes and pathways that are aberrantly induced by haploinsufficient levels of *CUX1*. These results are imperative to identify potential targets for novel therapeutics for -7/del(7q) myeloid neoplasms. The research group expects that completion of these studies will identify two roles for *CUX1*. First, *CUX1* is likely to function as both a critical regulator and transcriptional partner of essential myeloid transcription factors that drive differentiation. Second, haploinsufficient *CUX1* may be found to induce proliferative
genes and/or self-renewal pathways, which will facilitate subsequent research aimed at developing therapeutics to target these pathways.


Project 2 – Understanding the Leukemia Stem Cell
Team: Cunningham, Le Beau, White
Goal: Identify molecular pathways involved in the production of healthy blood cells.

Delineating the core components of the gene regulatory networks, or molecular circuitry, of leukemia stem cells and their normal counterparts is critical to the development of disease-specific drugs that are curative in therapy-related AML. The studies in this project, initiated by Drs. Singh and Cunningham, and now continued by Cunningham following the departure of Singh from the University of Chicago, have sought to take a novel approach to understanding these networks. Building on the investigators’ expertise in understanding the role of nuclear architecture, chromatin structure, and gene organization in normal and malignant stem cell populations, several mouse models have been established to study the mechanisms that are dysregulated in leukemia. The initial plan to focus on the role of two central factors, EZH2 and PU.1, has been expanded to study the role of the Jumonji family of histone modifying enzymes. Current studies in this area are funded by a grant from the Hyundai Foundation, and are using the cord blood transplant model (described in Project 4) to test the effect of dysregulation of KDM1, KDM2 and ARID5.

Dr. Cunningham’s laboratory has continued to examine the nuclear architecture and the organization of genes and other structures within the nucleus of cells. Having overcome problems associated with small numbers of cells through a collaboration with investigators in Australia, Dr. Cunningham is now using high-throughput DNA sequencing (ChIP-seq), FAIME (see Project 6), and RNA polymerase factory studies to study the interactome in normal and
malignant stem cells. Additional funding for this project is being sought from the National Cancer Institute.

**Project 3 – Pharmacogenetics: How to Predict Response to Therapy**

**Team:** Onel, Dolan, Huang, Gurbuxani

**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) associated with sensitivity to chemotherapeutic agents (e.g., cytarabine, clofarabine, daunorubicin, etoposide) used to treat AML and t-AML. Over the past year, they have developed cell-based models for the discovery of genotype-phenotype relationships. For cytarabine, they have taken those findings and evaluated them in a clinical trial. Their ultimate goal is to identify patients who are likely to have an adverse reaction or non-response to drug prior to treatment.

**Comprehensive Genetic Analysis of Diverse Populations Identifies Cytarabine Susceptibility Polymorphisms**

Resistance to cytarabine therapy, which is used to treat AML, is a common reason for treatment failure. Drs. Dolan and Huang used a whole-genome approach to investigate the genetic determinants of cytarabine-induced cytotoxicity. They performed a meta-analysis of SNP genotypes from genome-wide association studies involving 523 lymphoblastoid cell lines (LCLs) from individuals of European, African, Asian, and African American ancestry. Several of the highest-ranked SNPs were within the “mutated in colorectal cancers” (*MCC*) gene. *MCC* expression was induced by cytarabine treatment, increasing from 1.7 to 26.6 fold in LCLs.

Thirty three SNPs ranked at the top of the meta-analysis (*p* < 10⁻⁵) were successfully tested in a clinical trial of patients randomized to receive low-dose or high-dose cytarabine plus daunorubicin and etoposide; of these, 18 showed association (*p* < 0.05) with either cytarabine IC₅₀ (a measure of drug effectiveness at which the concentration shows the half maximal inhibitory effect) in leukemia cells or clinical response parameters (minimal residual disease, relapse free survival, treatment-related mortality). This count (n=18) was significantly greater
than expected by chance (p=0.016). They observed that for the rs1203633 SNP, LCLs with the AA genotype were more sensitive to cytarabine-induced cytotoxicity (p = 1.31 x 10^{-6}). In addition, the AA (versus GA or GG) genotype was associated with poorer overall survival (p = 0.015) and was likely a result of greater treatment-related mortality (p = 0.0037) in AML patients.

They also determined that carriers of the TT allele of rs2897047 (near the IRX2 gene) have a greater incidence of relapse and greater disease burden at day 22 after initiation of therapy, as determined by the detection of minimal residual disease. These observations are consistent with their findings in LCLs that cells with the TT genotype were more resistant to cytarabine.

Drs. Dolan and Huang plan to further validate the function of these genetic markers and replicate their findings in another clinical trial.

**Evaluating Genetic Determinants of Clofarabine in Lymphoblastoid Cell Lines derived from Caucasians and African Americans**

Clofarabine, a purine nucleoside analog, is used in the treatment of hematologic malignancies and as induction therapy for stem cell transplantation. The discovery of pharmacogenomic markers associated with chemotherapeutic efficacy and toxicity would greatly benefit the utility of this drug. Drs. Dolan and Huang sought to identify genetic and epigenetic variants associated with clofarabine toxicity using an unbiased, whole genome approach. They employed International HapMap lymphoblastoid cell lines (190 LCLs) of European (CEU) or African (YRI) ancestry with known genetic information to evaluate cellular sensitivity to clofarabine. Additionally, they measured modified cytosine levels to ascertain the contribution of genetic and epigenetic factors influencing clofarabine-mediated cytotoxicity.

Association studies revealed that 182 SNPs and 159 modified cytosines were associated with cytotoxicity in both populations at the threshold of p ≤ 0.0001. Correlation studies between cytotoxicity and baseline gene expression revealed 234 genes at p ≤ 0.05. Of these genes, six were implicated as having: (1) their expression directly correlated to cytotoxicity; (2) a targeting SNP associated with cytotoxicity; and (3) a local modified cytosine associated with gene
expression and cytotoxicity. Figure 2 illustrates their research approach. They identified a set of 3 SNPs and 2 CpG (modified cytosine) sites targeting these six genes, explaining 39.7% of the observed variation in phenotype. siRNA knockdown of the top three genes (SETBP1, BAG3, KLHL6) in LCLs revealed altered susceptibility to clofarabine, confirming their relevance in drug response.

This successful, comprehensive approach used by Drs. Dolan and Huang can be applied to find the genetic determinants of other drugs or cellular phenotypes. In future studies, they plan to collaborate with other team members (see Project 5) to test these SNPs and modified cytosines in a clinical trial that has utilized clofarabine.
**Project 4 - Using the Leukemia Stem Cell to Model Disease**  
**Team:** Cunningham, Le Beau  
**Goal:** Study t-AML using mouse models.

Dr. Cunningham has successfully established the immunodeficient mouse model of human t-AML. Specifically, he has demonstrated transplantability across 3+ generations of mice, each generation requiring 8-12 weeks of observation. These results indicate the successful engraftment of human leukemia stem cells (LSCs). He has also established several other acute myeloid and lymphoblastic leukemia mouse strains that are important not only for comparative purposes, but also serve to stimulate investigation in critical areas of leukemogenesis with other CRF-funded investigators.

Specifically, this model has been instrumental in the studies of the White laboratory in establishing the role of CUX1 in AML pathogenesis. Using a cord blood-based transplant model, Drs. Cunningham, White, McNerney, and Le Beau have collaborated on a seminal study that confirms the role of CUX1 in t-AML. The success of these experiments has established the feasibility of the approach, and forms the basis of an application to the Hyundai Foundation for additional funding.

In a related series of studies, the team is using the same model to develop novel therapeutics for t-AML. Working with Drs. Geoffrey Greene (Ben May Department for Cancer Research), as well as Dr. Matthew Tirrell (Director, Institute for Molecular Engineering), they are testing the feasibility of targeted killing of leukemia blasts in vivo using programmed cell death. Briefly, Dr. Tirrell, an internationally recognized biomaterials expert, has developed a micelle-based technology that can be targeted to specific cell populations. The micelle is novel given its hybrid nature, incorporating a surface antibody tag for targeting purposes, a molecular toxin which induces cytotoxic holes in the cell surface, and a micellar scaffold which allows stable and specific delivery of the toxin to the target cell. These studies are ongoing and are the subject of a grant application to the Rally Foundation.
Based on the long history of the research team in characterizing the clinical and cytogenetic characteristics of t-AML, and the recent progress they have made in determining the genomic profile of this disease (see Project 1), Dr. Le Beau has undertaken the development of mouse models that recapitulate the genetic changes in t-AML with a del(5q). In other studies, Dr. Le Beau’s laboratory has determined that the EGR1 and APC genes on chromosome 5 are likely to be involved in the pathogenesis of t-AML with a del(5q). Given the close association of TP53 mutations in t-AML with a del(5q), she hypothesized that mice that are genetically engineered to contain only one copy each of the EGR1 and APC genes (referred to as Apc \( ^{del/+} \) Egr1 \( ^{+/-} \)-mice) with decreased or absent Tp53 expression may maintain an expanded pool of aberrant stem/progenitor cells and contribute to the development of myeloid disease.

To test this hypothesis, Dr. Le Beau generated Apc \( ^{+/-} \) Egr1 \( ^{+/-} \)-mice that have a knock-down of Tp53 expression in hematopoietic cells, using shRNA technology. These studies revealed that decreased Tp53 expression contributes to the development of myeloid disease in a transplantation model when Apc and Egr1 are haploinsufficient. To date, 20% of mice transplanted with Apc \( ^{del/+} \) Egr1 \( ^{+/-} \) BM transduced with Tp53 shRNA have developed myeloid neoplasms. The investigators are currently performing a genomic profile of AMLs in these mice, which will be contrasted with the genomic profile of human t-AMLs generated (see Project 1). This mouse model of t-AML should reveal novel therapeutic targets for future exploitation, and will be a valuable resource in the future to test targeted therapies, as well as to examine the mechanisms by which t-AMls become resistant to treatment.

**Project 5 - Targeted Clinical Trials**

**Team:** 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.

**5-Hydroxymethylcytosine Alterations in Myeloid Malignancies**

From a basic science perspective, Dr. Godley has been investigating how covalent cytosine modifications mediate normal and malignant human hematopoiesis. As a result of her work
over the past year, she has begun to address whether these cytosine alterations can be used to predict responsiveness to hypomethylating agents, a promising new therapy for AML.

Epigenetic alterations, including histone modifications and DNA methylation (5-methylcytosine, 5-mC), are central to cellular differentiation and gene expression. Drugs thought to act as hypomethylating agents, namely 5-azacitidine and decitabine, are used clinically for patients with myeloid malignancies throughout the world, although the precise molecular mechanism by which they function is not known. Little insight exists in understanding why some patients will achieve remission or why the drugs stop working in patients who show an initial response.

Dr. Godley’s laboratory has demonstrated that these drugs result in increased levels of 5-hydroxymethylcytosine (5-hmC), a recently discovered covalent cytosine modification that arises through hydroxylation of 5-mC via the enzymatic activity of the TET proteins. They hypothesize that myeloid malignancies are driven, at least in part, through alterations in distribution of 5-hydroxymethylcytosine, which can be reversed by treatment with hypomethylating agents.

To address this hypothesis, they worked closely with Drs. Toyosi Odenike and Wendy Stock to perform correlative studies on peripheral blood and/or bone marrow samples in patients who have received 5-azacitidine as treatment for a myelodysplastic syndrome (MDS) or AML. Building on their experience with the high-dose cytarabine/mitoxantrone regimen published this year (Larson et al., 2012), the team now has an open clinical trial treating patients with 5-azacitidine followed by a high-dose cytarabine/mitoxantrone regimen (see next section for details). Samples from this trial, as well as others from previous similar trials using 5-azacitidine, are available to profile 5-hmC distribution before and after treatment. Previously, in collaboration with Dr. Chuan He, Dr. Godley developed a novel chemical labeling approach that allows simple identification of DNA loci enriched in 5-hmC specifically. This approach has been applied to some of the clinical trial samples. They are now analyzing preliminary sequencing data from this study. The results could reveal which patients are most likely to
respond to the hypomethylating agents, as well as the molecular mechanisms that underlie resistance to the drugs.

Over the past year, Dr. Godley’s laboratory has published several studies showing that 5-hmC levels are altered in myeloid malignancies. As expected from its enzymatic function of converting 5-methylcytosine to 5-hmC, myeloid malignancies with TET2 mutations have low levels of 5-hmC (Yamazaki et al., 2012 and Pollyea et al., 2013). They also contributed to work showing that some elderly patients with clonal hematopoiesis have acquired TET2 mutations and lack normal levels of 5-hmC (Busque et al., 2012). In the coming year, they anticipate that they will be able to describe the 5-hmC profiling of TET2-, IDH1-, and IDH2-mutant AML and t-AML.

In addition, Dr. Godley has continued to perform translational studies of patients with inherited bone marrow malignancies. Her laboratory identified a new family with dyskeratosis congenita by studying normal allogeneic stem cell donors who were found to be thrombocytopenic (Churpek, et al., 2012). They have also written clinical guidelines for these patients (Churpek, et al., 2013).

**New Therapeutic Regimen for High Risk Leukemias**

During the past year, Drs. Stock, Odenike, Godley, and Larson activated a novel trial that builds on their previous clinical and translational work in t-AML, which evaluates the potential benefit of adding a hypomethylating agent, 5-azacytidine, to an established regimen for high risk leukemias. The team previously published the effectiveness of this regimen in a retrospective analysis of high risk leukemias (Larson et. al, 2012). This regimen combines high dose cytarabine with mitoxantrone in a relatively non-toxic combination that results in high remission rates of over 50% with low treatment-related mortality of approximately 6% in these very high risk patients.

In their new regimen, based on in vitro modeling data from the Stock and Dolan laboratories using leukemia cell lines, as well as a recently published clinical trial from investigators at
Cornell, the team hypothesized that the addition of 5-azacytidine, a hypomethylating agent, would sensitize leukemia cells to the sequential administration of high dose cytarabine and mitoxantrone. The trial is a Phase I study designed to evaluate the safety of this combination with a dose expansion cohort to evaluate biological efficacy (testing of the hypothesis) at the maximally tolerated dose of 5-azacytidine. Activated in September 2012, the team has already completed enrollment of the first cohort (7 patients have been enrolled out of an expected total of 25 patients). To date, the new regimen has been well-tolerated and does not appear to result in more toxicity than the standard high dose cytarabine and mitoxantrone regimen. The study is accruing well (enrollment for the second dose cohort is ongoing), and enrollment is expected to be complete by the end of 2013.

Concurrent with the clinical trial, the team is collecting and banking bone marrow aspirate samples collected from patients prior to treatment, after 5 days of treatment with 5-azacytidine (prior to high dose cytarabine and mitoxantrone), and at the time of recovery of the white blood cell count to evaluate methylation and gene expression changes in key regulatory and drug metabolizing pathways. They plan to examine genes responsible for the activation of ara-c and anthracyclines, which are hypothesized to be upregulated in response to the hypomethylating effects of 5-azacytidine. These genes include ribonucleotide reductase, topoisomerase II, and deoxycytidine kinase.

Based on recent data from University of Chicago collaborator Dr. Jianjun Chen, which demonstrates the importance of specific micro-RNAs in the regulation of intracellular signaling in therapy-resistant leukemias, the team also plans to evaluate the role of 5-azacytidine in expression of the micro-RNA 129b. Drs. Stock, Odenike, and Larson are also collaborating with Dr. Jane Churpek at the University of Chicago to evaluate the family histories of enrolled patients and to collect germ line tissue for future genome-wide analyses. These efforts will facilitate the identification of genetic risk factors for t-AML.


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**Project 6 - Computational Biology – Developing an Atlas of Therapies**

**Team:** Lussier and 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.

During the early phases of this project, Dr. Lussier developed a new methodology, Functional Analysis of Individual Microarray Expression (FAIME), to help translate a patient’s gene expression signatures into molecular functions and pathways. The methodology computes the normalized centroid of each mechanism-anchored gene set based on rank-weighted gene expression of an individual sample. This approach is advantageous because it dramatically reduces the number of patients required for validation as compared to conventional gene expression signature classifiers that generally require a few hundred patients. Over this past year, Dr. Cunningham’s laboratory has been using FAIME to study the nuclear architecture in both normal and malignant stem cells (see Project 2). As a result of Dr. Lussier’s departure from the University, he was unable to complete the development of the planned Atlas of Therapies.
Challenges and opportunities

The interdisciplinary structure of the t-AML initiative has facilitated the genomic profiling of this disease, particularly the rapid transfer of data between laboratories. Examples include the exchange of expertise on genotyping platforms, and high-throughput sequencing platforms that accelerated the conduct of this work. Other examples include the exchange of expertise that informed Dr. McNerney’s and White’s work identifying CUX1 as a haploinsufficient myeloid suppressor gene on 7q. In particular, clinical expertise and knowledge of the individual patients (Drs. Larson, Stock, Godley, and Odenike), as well as cytogenetic data contributed by Dr. Le Beau informed the data analysis and interpretation. Once Dr. McNerney had made her initial observations of the cryptic focal deletions on 7q encompassing the CUX1 gene, and the fusion gene in an additional patient, Dr. Le Beau was able to provide validation of the loss of CUX1 using an independent assay on chromosomes from these patients (fluorescence in situ hybridization), Drs. Cunningham and Le Beau shared their expertise in modeling Cux1 loss in the mouse, and Dr. Cunningham used the mouse technologies he developed in Project 4 to provide the critical data necessary for publication. The investigators in this interdisciplinary project benefited from monthly meetings, as well as numerous dialogues and research discussions throughout the project period sparked by the team’s integrated research goals and collegial atmosphere.

The major challenge faced by the group was the departures of Dr. Singh and Lussier from the University, which necessitated long-distance collaborations and, in some cases, winding down certain components of the research.

Conclusion

The integrated leukemia program has accelerated the characterization of t-AML and treatment of this disease by bringing together a cadre of researchers with diverse expertise to focus on this poorly understood disease. Major discoveries and impact of the research program include the following:
• Rapid conduct of genomic profiling of t-AML, and identification of cellular pathways that cooperate in the pathogenesis of this disease;
• Identification of the first haploinsufficient myeloid suppressor gene on chromosome 7 involved in the pathogenesis of myeloid neoplasms, including t-AML;
• Accelerated development of innovative clinical trials, including a new Phase 1 therapeutic regimen for high-risk AML combining high dose cytarabine and mitoxantrone, with the hypomethylating agent, 5-azacytidine;
• The identification of genetic variants that influence the response to drugs used to treat AML, a critical step in personalizing cancer therapies;
• Creation of infrastructure for banking both germline and malignant tissues from t-AML patients, which will continue to be a valuable resource for UCCC researchers;
• Development of bioinformatics platforms for high throughout sequencing and transcriptome sequencing, thereby benefitting the Institute for Genomics and Systems Biology's 1000 Cancer Genome Project at UCM;
• Development of a new methodology facilitating the translation of a patient's gene expression signatures into molecular functions and pathways, namely Functional Analysis of Individual Microarray Expression (FAIME); and
• The launch of new technologies on campus, such as NSG mice to develop patient-derived xenografts models.

The impact of this program extends far beyond the work summarized in this report. For example, ongoing data mining of the genomic profiling data will be used for the identification of therapeutic targets, and development of clinical trials targeting the cellular pathways that are deregulated leading to t-AML. The mouse models generated by the investigators are valuable resources for preclinical studies, particularly screening of small chemical libraries to identify new compounds that inhibit the growth, or induce cell death, of the leukemia initiating cells, as well as studies to elucidate the mechanisms of drug resistance and disease progression. The investigators in the CRF Integrated Leukemia Program continue to interact and meet regularly.
Under the auspices of this interactive Program, new collaborations have been initiated with other University investigators, including Matthew Tirrell in the Institute for Molecular Engineering to induce targeted killing of the leukemia cells. Finally, research conducted over the past three years has formed the basis for a number of new grant applications to extend our work in t-AML. Selected examples include:

- Drs. Le Beau, Stock, and Dolan are leading a project in a Specialized Center of Research proposal to the Leukemia and Lymphoma Society to extend genomic profiling studies of t-AML, in the context of clinical trials, and to elucidate the mechanisms of resistance to therapy;
- Dr. McNerney, a junior faculty member, has submitted a K08 mentored research grant to the NCI for early stage investigators. Drs. White and Le Beau will serve as her co-mentors;
- Drs. Cunningham, Tirrell, and Greene are submitting a grant application to the Rally Foundation to examine the feasibility of targeted killing of leukemia blasts using novel micelle-based technology; and
- Dr. Cunningham has submitted an application to the National Cancer Institute to extend his studies of the nuclear architecture and organization of genes and other structures in normal hematopoietic stem cells and how they are disrupted in leukemia stem cells.

We are grateful for the support of the Cancer Research Foundation, which has enabled us to take a 'Team Science' approach to gain a global perspective on the biology of t-AML, an aggressive and deadly form of leukemia, using system biology technologies.