

WeCan CR-UK CRF Application: **Whole genome analyses of epigenetic therapies for AML.**

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### **Abstract.**

Acute Myeloid Leukemia (AML) is one of the most common hematopoietic cancers and despite intensive research there has been little change in survival from this disease in the past twenty years (1). The proposed studies extend ongoing whole-genome state-of-the-art epigenetic and expression analyses, in both cell lines and primary patient-derived AML samples, and are designed to better understand the mechanism of action of “epigenetic therapies” in AML.

### **Introduction.**

The impact of aberrant epigenetic programming on AML disease pathophysiology is increasingly recognized (2). Importantly, in contrast to genetic mutations, epigenetic aberrations are potentially reversible and so look to be an appealing therapeutic target. Both DNA methylation and histone modifications contribute to epigenetic programming. For example, there is growing evidence to suggest that dysregulation of histone methylation contributes to AML via specific mutations which dysregulate histone methyltransferases (HMTs). The PML-RAR $\alpha$  gene translocation-derived fusion protein, found in a form of AML (Acute Promyelocytic Leukemia (APL)), recruits the histone methyltransferase EZH2 which represses expression of differentiation-promoting genes, by trimethylation of lysine 27 on histone H3 (H3K27Me3) (3).

Because of these epigenetic alterations in AML, there is considerable interest in epigenetic therapies to combat this disease; for example, DNA methylation inhibitors (e.g. azacytidine (AzaC)) and EZH2 inhibitors (4). Preliminary epigenetic drug regimens have achieved between 30-60% hematological responses in published trials (5). Significantly, different epigenetic therapies appear to have a synergistic effect when used in combination (5). As a result, AzaC was recently approved for patients with myelodysplastic syndrome, AML with multilineage dysplasia and advanced chronic myelomonocytic leukemia, by the National Institute for Health and Clinical Excellence (NICE) and the Scottish Medicines Consortium (6).

Epigenetic modifying drugs have been postulated to function by reactivating tumor suppressor genes by removing the repressive effects of DNA and chromatin respectively (4). However, the relationship between AzaC-mediated DNA demethylation and clinical response is controversial, and it is now accepted that secondary effects are likely to contribute to drug activity (7). Consistent with this idea, there is abundant evidence suggesting that DNA methylation and histone methylation are mutually co-regulated (8, 9). Of particular interest in this regard, AzaC has been reported to suppress H3K27Me3 (10).

### **Hypothesis.**

We hypothesize that AzaC exerts its anti-neoplastic effects, in part, by cross-talking to histone methylation and countering dysregulation of histone methylation, specifically H3K27Me3.

To begin to test this hypothesis, Dr. Kirstin Lund, a University of Glasgow CRF jointly supervised by Dr. Mhairi Copland and Prof. Peter Adams, is performing an integrated epigenetic and gene expression analysis of myeloid cancer cells treated with and without AzaC.

Specifically, Dr. Lund is determining the genome wide distribution of DNA methylation (by Illumina 450K methylation array) and H3K27Me3 (by ChIP-seq) and gene expression (by RNA-seq), in AML3 cells treated with and without AzaC. In collaboration with Dr. Tony McBryan, a full time computer programmer in Prof. Adams lab, Dr. Lund will analyze the data to determine whether perturbation of DNA methylation affects H3K27Me3 across the genome and whether altered gene expression is spatially linked to inhibition of DNA methylation and/or H3K27Me3.

Dr. Lund is due to finish her lab work in October 2012 and submit her M.D. thesis by October 2013. This proposal seeks funding for another CRF to begin in October 2012, to extend this line of investigation. This will be achieved through the following two Specific Aims:

### **Specific Aims.**

**Specific Aim 1. To compare the effects of AzaC treatment on AML cells *in vitro* with the response to AzaC in patients.** Since AzaC is approved for treatment of AML (6), we will compare the effects of AzaC on AML cells *in vitro* with the effects of AzaC on AML blasts in

patients (*in vivo*). Specifically, we will use RNA-seq to compare changes in gene expression induced by AzaC *in vitro* and *in vivo*. The *in vitro* studies are currently being performed by Dr. Kirstin Lund. For the patient studies, we will isolate AML blasts from blood of AML patients collected before and after AzaC treatment. RNA will be collected and analyzed by RNA-seq, according to a standard protocol in the Adams lab. Data will be analyzed using an existing pipeline established by Dr. Tony McBryan in the Adams lab. Changes in gene expression *in vitro* and *in vivo* will be compared and contrasted to assess the concordance of the *in vitro* and *in vivo* changes, and develop a consensus AzaC gene expression response signature and linked topographical map of chromatin structure (from Dr. Lund's ongoing studies).

**Specific Aim 2. To compare the effects of AzaC, inhibition of EZH2 and the double-combination on AML cell gene expression and phenotype.** If AzaC exerts its effects via suppression of H3K27Me3, then direct suppression of H3K27Me3 should recapitulate at least some of the effects of AzaC on gene expression and cell phenotype. To test this, we will compare the effect of AzaC and direct inhibition of EZH2 on AML cell gene expression (by RNA-seq), proliferation and differentiation. In addition, we will assess the effect of combined AzaC and EZH2 inhibition. To inhibit EZH2, we will use siRNA knock down and, if available, EZH2 small molecule inhibitors in pre-clinical and clinical development. Several pharmaceutical companies (e.g. GSK, Novartis and Pfizer) are developing such inhibitors and we are currently in discussions with GSK to obtain one such compound. Proliferation and differentiation will be analyzed using standard assays routinely performed in the Copland lab. RNA-seq data and functional/phenotypic data will be analyzed to determine whether inhibition of EZH2 mimics effects of AzaC on gene expression and cell function, and whether there is an additive and/or synergistic effect of combined inhibition of DNA and histone methylation. The molecular basis of gene expression changes that are matching or additive/synergistic between AzaC and EZH2 inhibition will be investigated at the chromatin level, using gene-specific or whole genome (e.g. ChIP-seq) approaches that are standard in the Adams lab.

### Summary.

These studies will yield a better understanding of the mode of action of AzaC and other epigenetic therapies, alone and in combination. This will facilitate development of biomarkers to predict and monitor patient response and promote rational design of combination therapies.

### References.

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