THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOLOGY

Implication of Orphan Nuclear Receptors NR4A1 and NR4A3 in a Conditional Knockout Murine Model for Acute Myeloid Leukemia

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Abstract

Limited understanding of the mechanisms that control the proliferation of cancerous cells such as those found in acute myeloid leukemia (AML) is among one of the most important reasons why we do not know how to treat cancers appropriately. Further investigation into the way that cancers develop is our current hope in developing new treatments for disease. We show in this set of experiments that genes NR4A1 and NR4A3 are implicated in the onset of AML as shown previously[5]. A Tamoxifen induced conditional knockout murine model with floxed NR4A1 regions was used to show that the down regulation of both NR4A1 and NR4A3 leads to AML. Hematoxylin and Eosin staining was performed upon lung, liver, and spleen samples in order to view the myeloid infiltrates that are present in the conditional knockout as opposed to wild type tissues. Bone marrow was isolated from AML mice and transplanted into lethally irradiated mice. After transplantation, these mice displayed complete engraftment of the bone marrow cells and confirmed that this disease is transplantable. In addition, an NR4A3 rescue was attempted by isolating other samples of bone marrow from the AML mice and employing retroviral infection techniques. Using Fluorescence-activated cell sorting (FACS), we isolated upper and lower side population cells from wild type and conditional knockouts in order to perform a stem cell assay. The conditional knockout stem cell colony formations displayed a skewing of lineage potential for upper-primed cells. With these data, we detail novel findings in the shift of lymphoid-primed cells to a myeloid-like phenotype, suggesting that more research will be necessary to understand this breakdown in hematopoiesis.

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Introduction

Cancer is the second leading cause of death among Americans after heart disease[1]. Though typically seen in adults over the median age of 55, cancer can also afflict children in cases which account for 1% of the total amount of cases seen in the United States. Men are more prone to getting cancer (1 out of 2) while women are slightly less prone (1 out of 3). The causes of cancer are diverse, ranging from environmental factors and aging, to risky behaviors such as smoking and alcohol use or simply genetics.

An estimated 1,638,910 new cases of cancer are expected to be diagnosed in the year of 2012 alone[1]. Leukemia, or cancer of the blood and bone marrow, is estimated to account for approximately 3% of newly diagnosed cancer cases, which may seem like such a small percentage of the whole population affected. Upon further inspection, one can see that statistical representations gauge leukemia in particular to be on the rise of 0.5% each year since 1992, meaning that this cancer is on the rise in the overall population. Furthermore, since all leukemias are not created equally, treatment options are limited for some more than others.

Of the four major kinds of leukemia, acute myeloid leukemia (AML) has the worst prognosis; patients who receive a diagnosis of AML have a survival rate of less than 24%. Recent research suggests that abnormal chromosomal translocations are pertinent to targeting a variety of transcription factor genes that are involved in the formation of AML[3]. The disease begins as a breakdown in hematopoiesis, the pathway that contributes to the formation of blood cells in the human body (*Figure 1*).

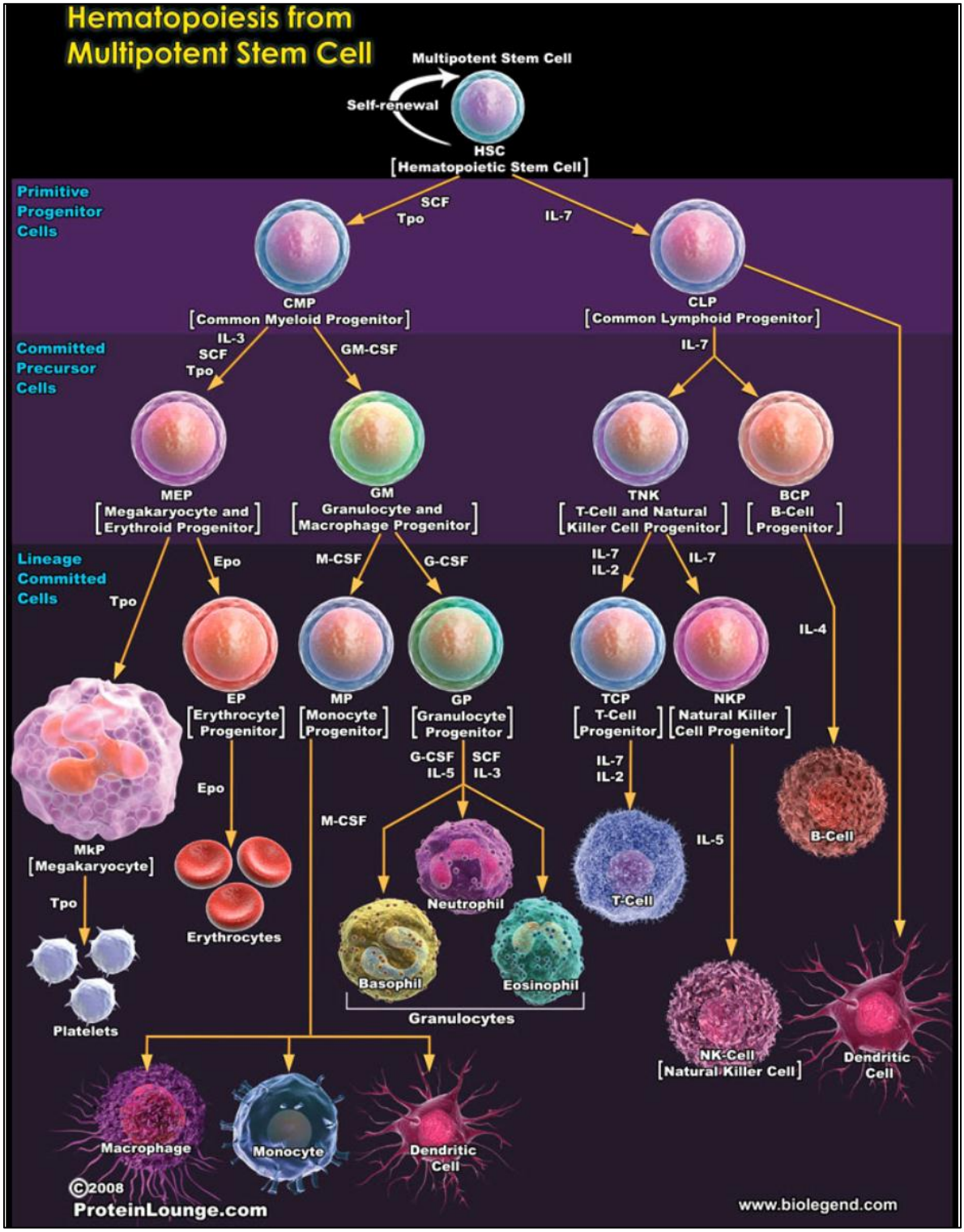

***Figure 1*:** Hematopoiesis leads to the differentiation of pluripotent hematopoietic stem cells (HSCs) into progenitors of a myeloid or lymphoid variety. Cells then further differentiate into a variety of white blood cells and red blood cells in downstream pathways. Source: biolegend.com.

Hematopoiesis is initiated in an embryo during the first few weeks of development and spreads to the organs of the liver and the spleen. Once the hematopoietic stem cells (HSCs) have begun to proliferate at a pace suitable to proper growth, the developing bone marrow takes over and becomes the central place in the human body in which hematopoietic cells may arise.

Every blood cell in the human body arises from HSCs because of their pluripotent nature. The differentiation of HSCs into blood cells occurs with a split into two possible pathways: the cells will either become lymphoid or myeloid in nature. HSCs are responsible for the initial differentiation into myeloid or lymphoid progenitor cells, which means that they are targeted to become specific kinds of blood cells with specialized functions. Of the lymphoid variety, the possible cells that can be formed include plasma cells, B cells, CTLs, T cells, and T helper cells of both types 1 and 2. Of the myeloid type, the cells formed include neutrophils, macrophages, eosinophils, erythrocytes, megakaryocytes, mast cells, and basophils. The signaling mechanisms that trigger the formation of these cells are very complex and not well understood, but it is known that formation of certain kinds of cells is caused by signaling and transcription factors.

Acute myeloid leukemia arises from a breakdown in the proper differentiation of myeloid cells, leading to the formation of undifferentiated blasts (*Figure 2*). Diagnosis of AML does not occur until a patient has reached a blood cell count of 20% blasts in their system. Since these blasts are free floating in the blood stream, it is in their nature to infiltrate healthy tissues in the human body, primarily the liver and spleen, until it has crowded out healthy cells necessary to fight infection and to function properly. The concentration of cells in these areas of the body leads to a swelling of these organs known as hepatomegaly and splenomegaly, respectively (*Figure 3*). This crowding effect can lead to a primary symptom of anemia in AML patients, along with other symptoms such as fatigue, bruising, paleness, and weight loss[6]. Since these

symptoms can often be attributed to many other minor diseases, patients with AML and leukemia in general do not typically get diagnosed until the disease has progressed for quite some time.

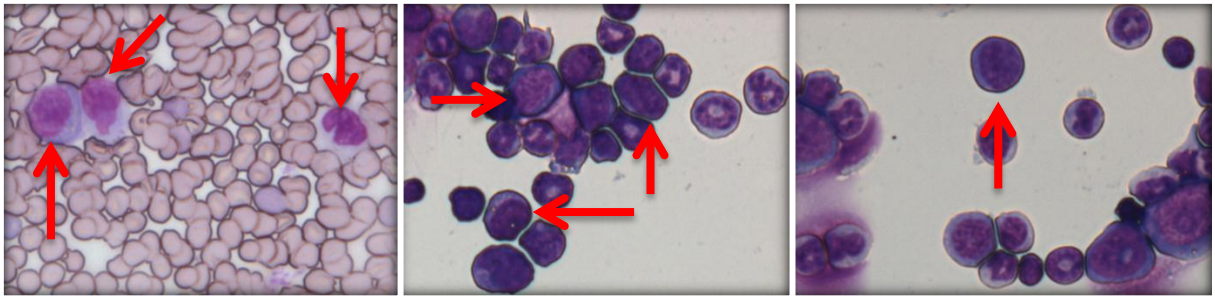

***Figure 2*:** Undifferentiated myeloid cells known as blasts are present in mice afflicted with AML. These cells are identified by red arrows and are shown in (A) the peripheral blood, (B) bone marrow, (C) and spleen. Source: Ramirez-Herrick AM et al., 2011.

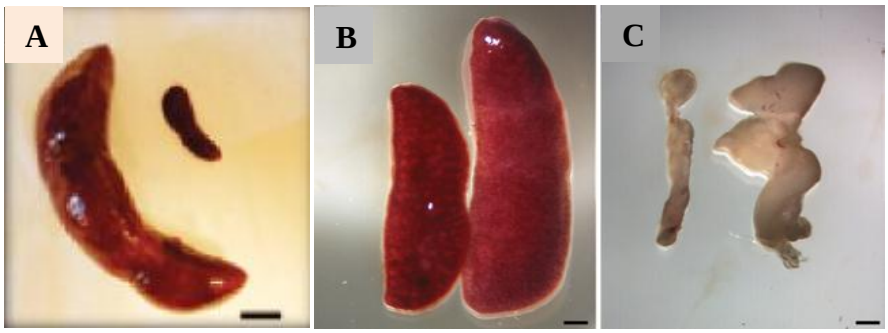

***Figure 3*:** Tissue swelling in AML afflicted mice. (A) and (B) display splenomegaly, and (C) displays swelling of the lymph nodes. Sources: Mullican SE et al., 2007; Ramirez-Herrick AM et al., 2011.

AML has a median age of development in individuals over the age of 65 and is more prevalent in men[6]. Current treatments for the disease rely upon chemotherapeutic methods with drugs such as Gleevec or Daunorubicin which are typically used to treat less serious forms of

leukemia such as chronic myeloid leukemia (CML)[2]. Patients may also undergo bone marrow transplants, all-trans retinoic acid treatments, or a variety of new clinical trial treatments that have not reached the mainstream pharmaceutical market. A lack of proper diagnosis and treatment for AML stem back to our lack of understanding of the mechanisms of its development and is the reason why we must pursue research projects that focus upon dismantling the disease.

Recently, the Conneely lab at the Baylor College of Medicine in Houston, Texas discovered the implication of two genes in the development of AML[5]. Orphan nuclear receptor genes NR4A1 and NR4A3 are down regulated in human patients with AML (*Figure 4*). These nuclear receptors are known to be triggered by a variety of growth factors, neurotransmitters and cytokines and are speculated to influence a variety of differentiable, apoptotic and proliferative effects within cells (*Figure 5*).

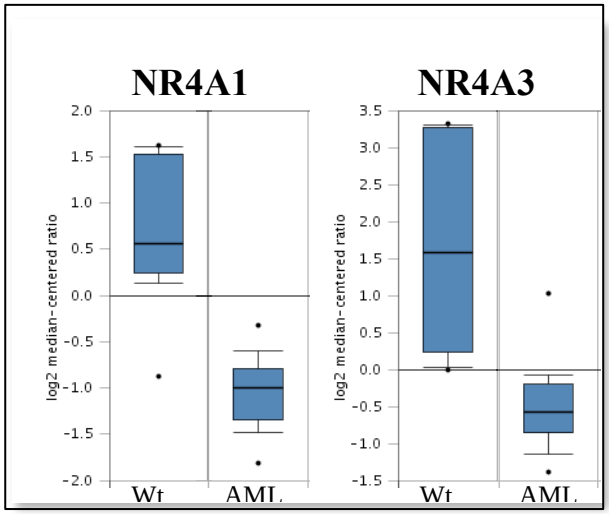

***Figure 4*:** Both orphan nuclear receptor genes NR4A1 and NR4A3 are down regulated in human patients suffering from acute myeloid leukemia. Source: oncomine.org.

Confirmation of the lethality of the down regulation of these genes was shown in a double knockout (DKO) model $Nr4a3^{-/-}Nr4a1^{-/-}$ (*Figure 6*). DKO mice showed progression to AML both phenotypically and at a cellular level (*Figure 7*, *Figure 8*). Pups died after two to four weeks with hunched posture, ruffled fur, and in smaller stature as compared to their healthy littermates. After this model was evaluated, a hypoallelic model consisting of $Nr4a1^{+/-}Nr4a3^{-/-}$ and $Nr4a1^{-/-}Nr4a3^{+/-}$ mice was created and was shown to lead to chronic myeloid malignancies[7]. Hypoallelic mice showed poor prognosis over a longer time frame than DKO mice and did not develop AML (*Figure 9*).

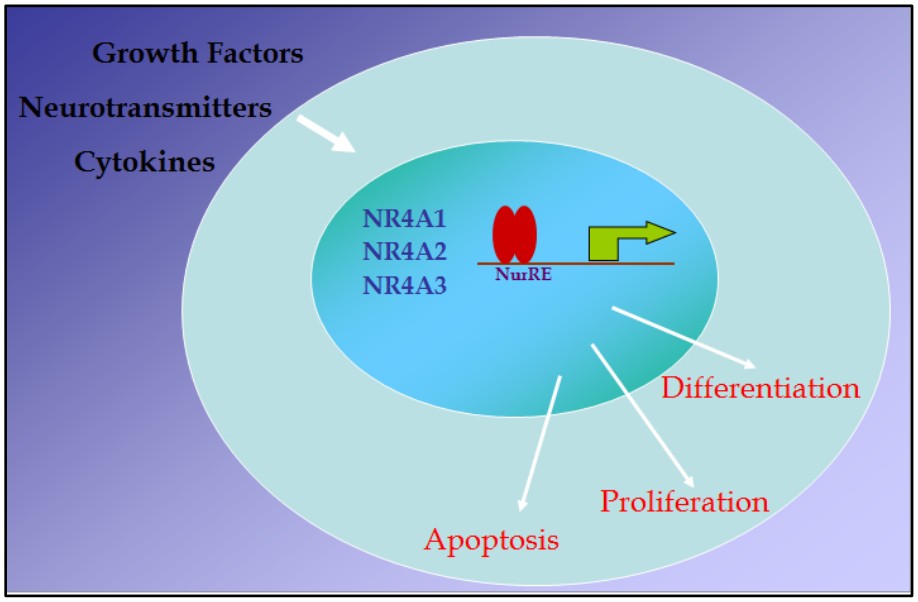

***Figure 5*:** Members of the orphan nuclear receptor family NR4A are influenced by growth factors, neurotransmitters and cytokines and elicit apoptotic, proliferative and differentiable effects within cells. Nuclear receptors activate genes at their respective response elements (NurRE). Source: Ramirez-Herrick AM, 2011.

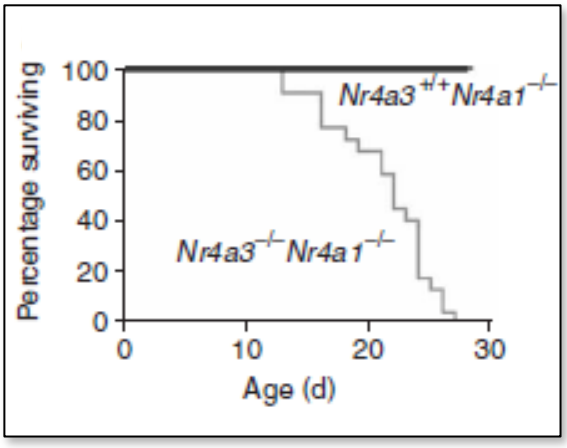

***Figure 6*:** The survival rates for DKO mice (n = 23) in comparison to $Nr4a3^{+/+}Nr4a1^{-/-}$ controls (n = 20). Source: Mullican SE et al., 2007.

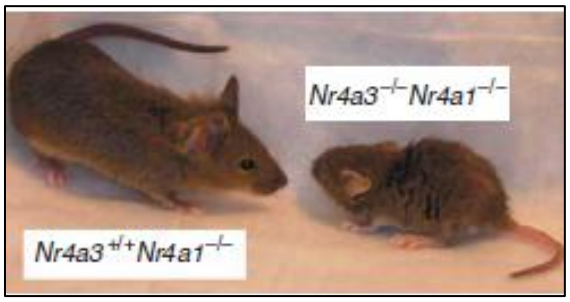

***Figure 7*:** DKO mice displayed hunched posture, smaller stature and ruffled fur in comparison to littermates. All DKO mice died by age 2-4 weeks. Source: Mullican SE et al., 2007.

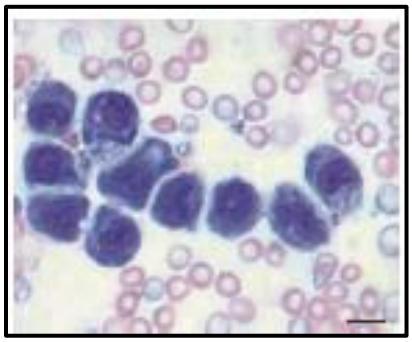

***Figure 8*:** Peripheral blood smear from DKO mouse age 2-3 weeks displaying ≥ 20% of cells are blasts. Source: Mullican SE et al., 2007.

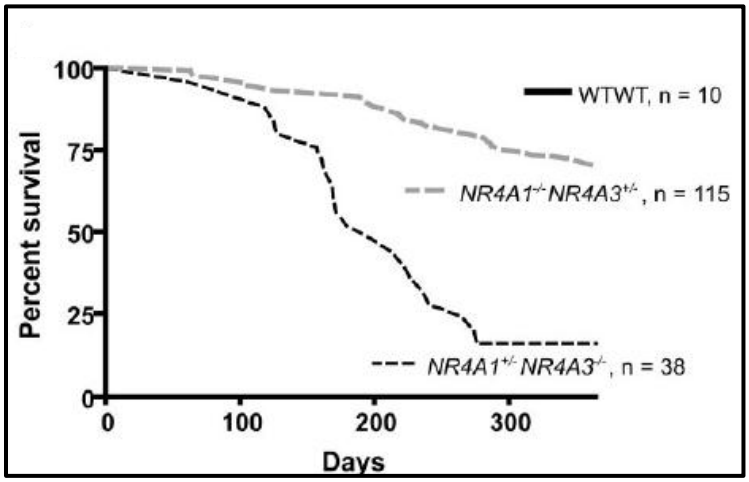

***Figure 9*:** Kaplan-Meier plot for the hypoallelic model versus wildtype littermates. Mice lacking both copies of Nr4a3 showed a poorer prognosis in comparison to lacking both copies of Nr4a1. Source: Ramirez-Herrick et al., 2007.

While the previous two models gave evidence to the importance of NR4A1 and NR4A3 in the prevention of hematopoietic chaos, it was hoped that a disease model could be created to recapitulate the human form of AML, which typically occurs later in life. A conditional knockout was generated using Cre recombinase-Estrogen Receptor mice crossed with $Nr4a3^{-/-}Nr4a1^{flx/flx}$ mice to generate Cre $Nr4a3^{-/-}Nr4a1^{flx/flx}$ mice (*Figure 10*). The conditional knockout was shown to be inducible by Tamoxifen by A. Ramirez-Herrick (not yet published).

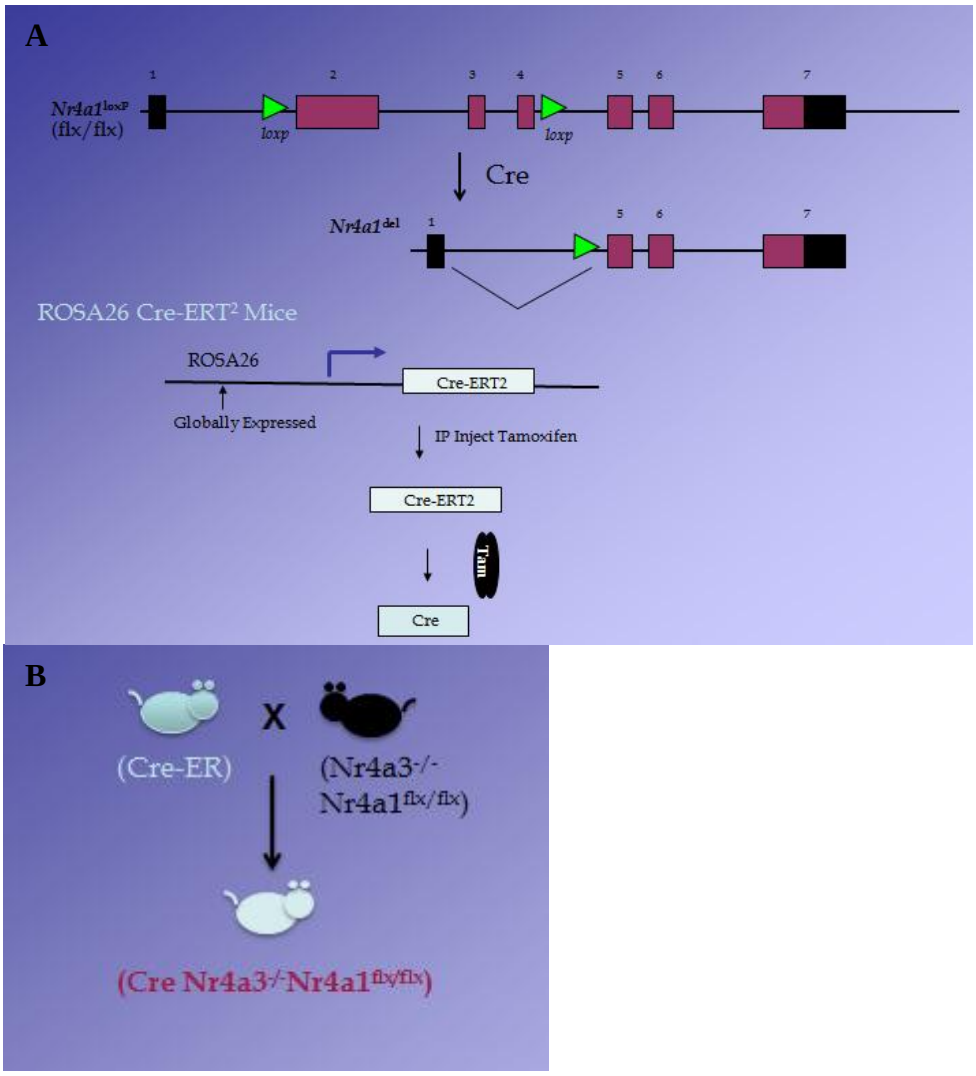

Figure 10: Conditional Knockout with Nr4a1 floxed sites. (A) The knockout was inducible using the Cre-recombinase system which is activated through Tamoxifen injection. Mice were generated containing the globally expressed ROSA-26 promoter. Upon intraperitoneal injection, Cre-ERT2 was activated to Cre recombinase, which activated the excsision of genetic material flush between loxp sites including loci 2-4 in the Nr4a1 gene. (B) The CK was generated with a Cre-ER mouse line and a mouse line containing $Nr4a3^{-/--}Nr4a1^{flx/flx}$. Source: Ramirez-Herrick AM, 2011.

With an established conditional knockout model that was hypothesized to lead to the development of AML, we posed a number of questions in the following experiments. First, we wanted to know if the conditional model would progress to AML and show evidence at a molecular level through staining procedures. Next, we hoped to estimate the origin of breakdown in the hematopoietic pathway using a stem cell assay to compare wild type and conditional knockout upper (lymphoid-primed) and lower (myeloid-primed) side population cells. Finally, we attempted a retroviral rescue of the NR4A3 gene in lethally irradiated mice in the hopes of a first step toward therapy for AML.

Histologic analyses showed that myeloid infiltrates are present in various non-hematopoietic tissues in affected mice through hematoxylin and eosin staining. We also showed that there may be a shift in lineage potential for rare, quiescent side population cells that comprise less than 1% of the total stem cell population. The nature of the shift appeared to be from a lymphoid-like state to a myeloid-like state, shown with colony forming unit data. The retroviral infection was not completed within the time frame of this research project and was finished by A. Ramirez-Herrick.

Methods

Staining:

Tissue samples were isolated from wildtype (WT) mice and Conditional Knockout (CK) mice (post-Tamoxifen injection) including the lung, spleen, kidney, lymph nodes, and liver. Additionally, blood smears and cytospins of bone marrow were obtained for myeloperoxidase staining and May-Grunswald staining (results not published). Isolated tissues were fixed in 4% paraformaldehyde for 24 hours at 4°C, and then stored in 70% EtOH. Samples were embedded in paraffin and cut into 5-μm sections and laid upon slides. Hematoxylin and eosin staining was performed using the following scheme: 3 x 3' xylene, 3 x 3' 100% EtOH, 3' 95% EtOH, 3' 80% EtOH, 3' 70% EtOH, 5' distilled water, 1' Hematoxylin Stain 1 Gill (Fisher), 10 dips in distilled water to rinse, 2' distilled water, 30 seconds 1X PBS, 3 dips Eosin/Phloxin Stain (Poly Scientific), 3 x 10 dips 95% EtOH, 3 x 5' 100% EtOH, and 2 x 5' xylene. Slides were left to dry overnight. Slides were viewed using an Olympus BX41 microscope and Olympus DP71 camera (Olympus). All images were acquired using the Olympus DP controller and processed using Adobe Photoshop Version 10.0 (Adobe Systems).

Confirming Genotypes of Mice:

Tail samples (0.2 – 0.5 cm) were acquired from WT and CK mice to ensure that proper mice would be used for bone marrow isolation. DNA was isolated from tails using an E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek). Samples were first analyzed for CRE presence by polymerase chain reaction (PCR) (CGG TCG ATG CAA CGA GTG AT, CCA CCG TCA GTA CGT GAG AT). Samples were also analyzed for presence of Nr4a3 gene by PCR. Solutions

were run on 1% agarose gels with 3 μL ethidium bromide. Gels were viewed under UV light and images were analyzed with Microsoft Paint (Microsoft).

Inducing the CK Model and Bone Marrow Isolation:

Tamoxifen mixtures were made by protocol with 25 mg of 4-OH and 25 mg of the racemic mixture (Sigma). CK mice were injected with the mixture for a period of five days to induce AML. Mice were then treated with 5-Fluorouricil (5 FU) to bring out rare quiescent stem cells. Mice were euthanized using Isothesia™, and dissected for legs and arms. Bones were flushed with Hanks Balanced Salt Solution (HBSS) and 5% Fetal Bovine Serum (FBS). Cells were filtered and centrifuged for 10 minutes at 1.5 x 10^3 rpm at 4°C. Supernatant was aspirated off and red blood cells were lysed using ACK Lysing Buffer (Quality Biologicals). Cells were again treated with HBSS/FBS and centrifuged as previously. Supernatant was removed and cells were brought up again in HBSS/FBS to be measured on a hemacytometer. Dilutions were performed and pre-warmed Dulbecco's Modified Eagle Medium was added to samples (Invitrogen). A control sample was created from a WT mouse, prepared as CK samples were.

Cell Sorting:

WT cells were treated with verapamil and Hoechst Red/Blue dye. CK cells were treated with Hoechst Red/Blue dye, only. Tubes were vortexed and left to incubate at 37°C for 90 minutes in dark conditions. MethoCult® Stem Cell Media pre-treated with IL-3, IL-6, and stem cell factor was heated to room temperature and spiked with penicillin/streptomycin (Stem Cell Technologies). The mixture was shaken vigorously and pipetted into eight 96-well plates. Plates were refrigerated until sorting. Cells were treated with propidium iodide (PI) prior to sorting in a dark environment. Bone marrow cells were sorted one per well of each plate with replicates of

plates for upper side population WT, lower side population WT, upper side population CK, and lower side population CK using a FACSAria (BD Biosciences).

Stem Cell Assay:

The stem cell assay was prepared as described in the previous section and then incubated at 37°C 5% CO_2 for 15 days. Colonies were scored at days 7, 11, and 15 using guidelines from MethoCult® procedures for colony forming assays (Stem Cell Technologies). Plates were evaluated using an Olympus BX41 microscope and Olympus DP71 camera (Olympus). All images were acquired using the Olympus DP controller and processed using Adobe Photoshop Version 10.0 (Adobe Systems).

Retroviral Infection of Nr4a3:

Replicate 6-welled uncoated retronectin plates were made with Dulbecco's Phosphate-Buffered Saline (DPBS) and retronectin. Plates were incubated, media was removed, and then 5% Bovine Saline Albumin (BSA) was added to each plate. Plates were then rinsed again with DPBS and plates were parafilmed overnight at stored at 4°C. The procedure was repeated to obtain two more retronectin plates.

293T cells were plated in a tissue culture 6-welled plate and transfected with green fluorescent protein (GFP) producing virus. Bone marrow cells isolated from CK mice previously treated with 5FU were added to retronectin plates and centrifuged at 1200 rpm for 10 minutes with no deceleration. Supernatant was removed and filtered virus was added to polybrene and mixed gently. This solution was then added to the bone marrow cells and centrifuged again at 1200 rpm for 45 minutes with no deceleration. Pre-warmed 10% FBS/DMEM was added to the 293T cells and plates were incubated. Plates were again spun down as before and fresh media

was applied prior to incubation. Virus was then collected from the 293T cells and filtered. Bone marrow cells were spun as before and virus was put on retronectin plates. Plates were treated as before with centrifugation and incubation and then Ethylenediaminetetraacetic acid (EDTA) was applied to the plates. Cells were collected in a tube by centrifugation and EDTA was removed. Finally, cells were brought up in stem cell media (treated as previously) and added to fresh plates with mixing.

Mouse serum was combined with cultured bone marrow cells that were sorted for GFP^+. To assist in the rescue, helper cells were isolated from the bone marrow of a WT mouse and combined with newly sorted GFP/CK cells. WT and NSG mice were lethally irradiated twice and cells were injected retroorbitally. Mice were observed for progression to AML or improvement in health due to rescue scenario.

Results

Staining:

Hematoxylin and Eosin staining confirmed the presence of myeloid infiltrates in the tissues of the lung and liver in induced CK versus WT (*Figure 11*, *Figure 12*). A complete effacement of the architecture of the spleen was noted in induced CK versus WT tissues (*Figure 13*). Myeloid infiltrates were also visible in the peripheral blood smear, bone marrow, and lymph nodes of the induced CK as compared to WT in myeloperoxidase and May-Grunswald slides (results not shown).

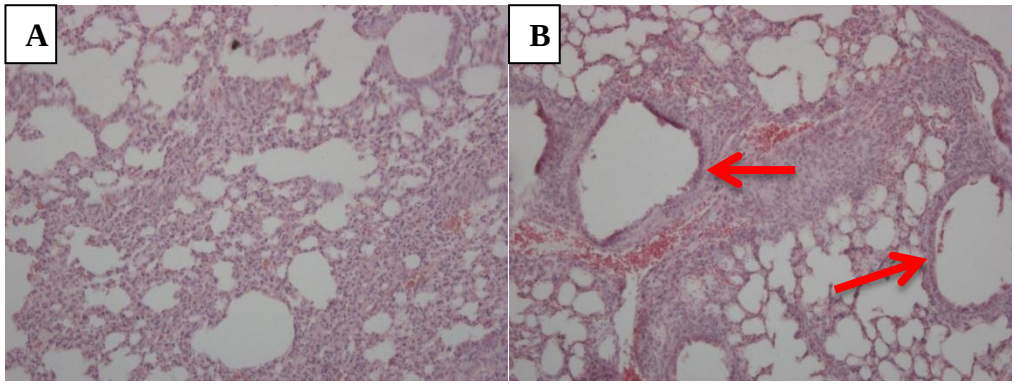

***Figure 11*:** Hematoxylin and Eosin stains of lung tissues from (A) WT tissue and (B) induced CK tissue. Myeloid infiltrates (purple, highlighted by red arrows) are visible along lumens of the CK tissue, but not the WT tissue. (Both images taken at 10X magnification.)

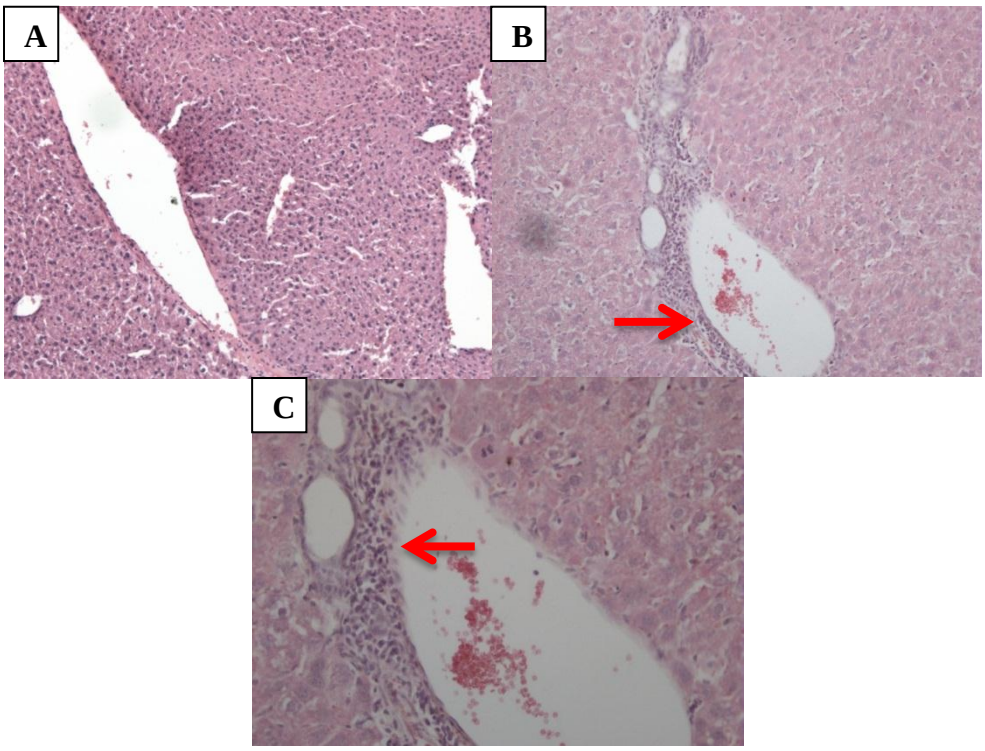

***Figure 12*:** Hematoxylin and Eosin stains of liver tissue from (A) WT tissue, and (B), (C) induced CK tissue. Myeloid infiltrates are visible (purple, highlighted with red arrows) around the blood vessel of the induced CK tissues and not the WT tissues. (Images (A) and (B) were taken at 10X magnification and image (C) was taken at 20X magnification.)

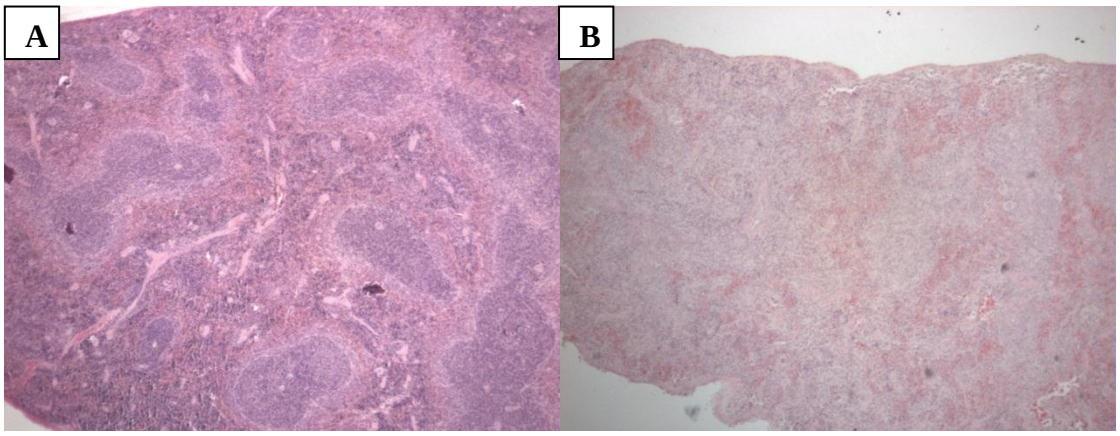

***Figure 13*:** Hematoxylin and Eosin stains of the spleen in (A) WT tissue and (B) induced CK tissue. Infiltrates in this tissue are not visible, but a complete effacement of the architecture of the spleen nodules is noted by red arrows highlighting their presence in WT tissue. (Both images were taken at 4X magnification.)

Confirmation of Genotype:

PCR results for genotyping were performed for every set of mice used in this experiment. A sample of Nr4a3 genotyping is provided below (*Figure 14*).

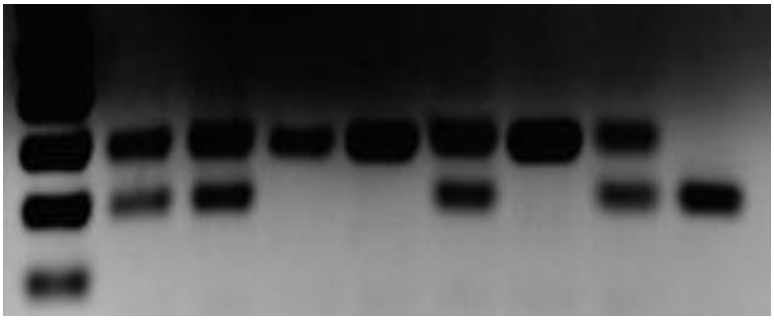

***Figure 14*:** PCR verification of the Nr4a3 genotype in experimental mice. Gel is comprised of 1% agarose with ethidium bromide spike. Two bands indicate +/- genotype, upper band only indicates +/+ genotype, and lower band only indicates WT.

Colony Forming Assay:

Counts were based upon the presence of colony forming units (CFUs) such as CFU-Ms and CFU-GMs as set out in the guidelines for MethoCult® Stem Cell Media (*Figure 15*). Plates that contained lower side population cells for both WT and induced CK displayed similar CFU-GM counts (*Table1, Figure 16*). Plates that contained upper side population cells for WT and induced CK differed in CFU-GM counts (*Table 2, Figure 17*). Data were based upon the average of the replicate plates together.

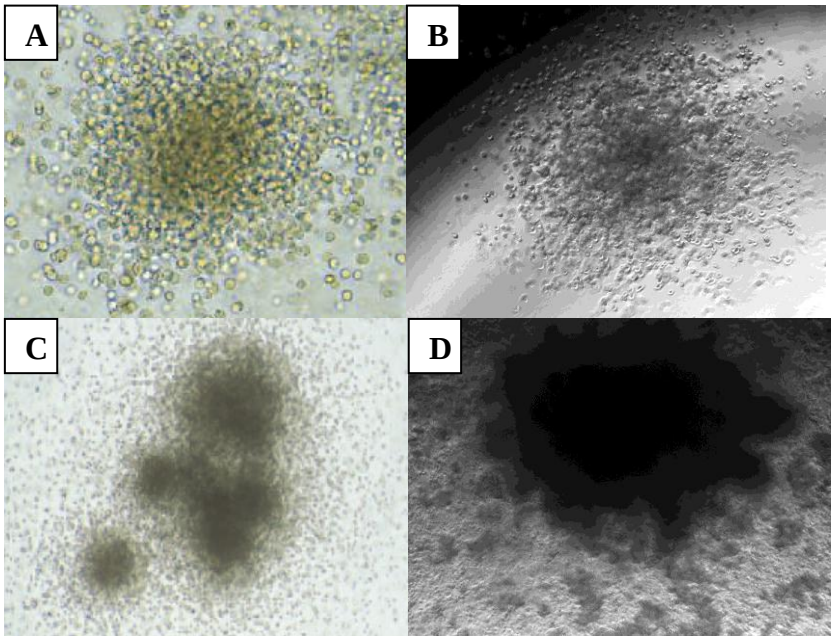

***Figure 15*:** MethoCult® Stem Cell Assay CFUs. (A) CFU-Ms as laid out by Stem Cell Technologies expected results. (B) CFU-Ms observed in experimental assay. (C) CFU-GMs as laid out by Stem Cell Technologies expected results. (D) CFU-GMs observed in experimental assay.

***Table 1*:** Lower Side Population CFU counts for WT and induced CK plates for days 7, 11 and 15 of assay. Replicate plate counts were averaged. Similar total CFUs were shown for both WT and CK cell types.

Lower	WT Total	WT CFU-GM	CK Total	CK CFU-GM
Day 7	29	**4**	30	**5**
Day 11	35	**9**	36	**9**
Day 15	36	**9**	36	**11**

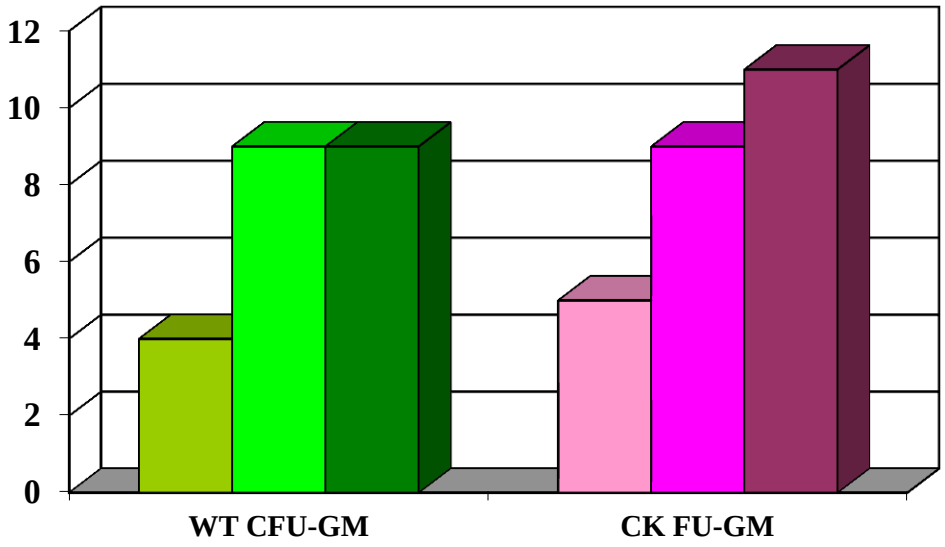

***Figure 16*:** Graphical representation of the comparison between WT and induced CK lower side population stem cells for CFU-GMs, only. Bars represent Days 7, 11, and 15, respectively.

***Table 2*:** Upper Side Population CFU counts for both WT and induced CK plates for days 7, 11, and 15 of the assay. Replicate plate counts were averaged. Cells formed similar numbers of total colonies, but differed greatly in CFU-GM quantities.

Upper	WT Total	WT CFU-GM	CK Total	CK CFU-GM
Day 7	33	7	34	13
Day 11	40	11	37	25
Day 15	37	11	43	23

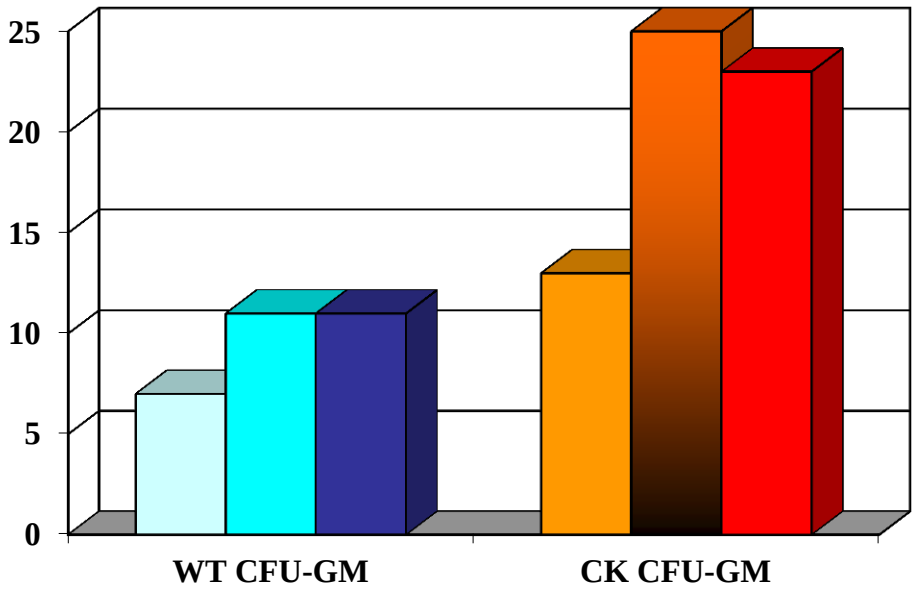

***Figure 17*:** Graphical representation of the comparison between WT and induced CK upper side population stem cells for CFU-GMs, only. Bars represent Days 7, 11, and 15, respectively.

Discussion

Staining indicated myeloid infiltrates in non-hematopoietic tissues for the induced CK:

Hematoxylin and Eosin staining is also known as the routine stain [8]. Most tissues are first put through this type of staining in order to obtain a visual of the nucleus of a cell and its present state of activity. This is helpful in the study of AML since the presence of blasts, or abnormally differentiated cells, is a hallmark of the disease. The stain is comprised of two dyes, one which stains the nucleus and the other which targets cytoplasm and connective tissues. Hematoxylin is dark purple in coloration and targets the nuclear portion of cells, while Eosin is orangish-pink and stains the rest of the material in the cell as a counterstain.

Staining requires the paraffinization of tissues before they can be sectioned and then put onto slides. This procedure is timely, but can render a great amount of information at a microscopic level. In this experiment, a progressive staining technique was employed in which tissues were hydrated slowly from their waxed state and then treated with Gill's hematoxylin. This experiment showed that there was a drastic difference between the tissues of a WT mouse versus those of an induced CK mouse. WT lung tissues showed no indication of the presence of myeloid infiltrates, but induced CK tissues showed the presence of myeloid infiltrates, clustered around lumens where blood vessels would have been in the living creature (*Figure 11*). Similarly, liver tissues for WT showed normal cells and no myeloid infiltrates while induced CK tissues displayed infiltrates clustered around lumens similar to those in the lungs (*Figure 12*). These samples in particular suggest that the infiltrates have traveled through the circulatory system to these non-hematopoietic tissues since the anatomical separation of liver, lung and bone marrow is apparent. The spleen samples show that loss of architecture results from the infiltration of blasts in induced CK tissues (*Figure 13*). This is another non-hematopoietic tissue

that is impacted by the induced CK model and further indicates that these animals have progressed to AML.

Upper Side Population Cells in the induced CK show a shift in lineage potential:

MethoCult® Stem Cell Assays are designed with a media that simulates the environment a cell would encounter *in vivo*, including a semi-solid media in which progenitor cells can begin to form colony forming units (CFUs). Supplemental reagents such as stem cell factor (SCF), various interleukins (ILs), and often antibiotics are combined with the media before it is pipetted into welled plates in order to ensure that the environment is as realistic for cells as possible, but also to prevent contamination. SCF in particular plays an important role in hematopoiesis, allowing for increased survival rate of HSCs and their continued proliferation. IL-3 is pertinent to the differentiation of HSCs into myeloid progenitor cells, so their use is of particular importance in the study of leukemic myeloid stem cell behavior. IL-6 assists in the detection of CFU-GMs from bone marrow stem cells by promoting progenitor differentiation.

A cell sorter is a special kind of machine that can differentiate between kinds of cells when a proper dye is implemented. For this set of experiments, a BD FACS Aria III was employed and used upon isolated bone marrow HSCs treated with Hoechst Dye. The purpose of using the dye was to sort for the side population, or rare stem cells, that comprise less than 1% of the total stem cell population. Within these side population cells is a mechanism that can pump out toxins and, likewise, dyes. Each primed lineage can also pump out toxins a bit differently, so two different dyes, Hoechst Red and Hoechst Blue, were utilized to sort for both the lower, or myeloid primed, and upper, or lymphoid primed, cells. Each cell was sorted into an individual

well containing the treated media and incubated at 37°C for fifteen days in order to observe the growth of the cell colonies into CFU-GMs and other colony formations.

Observation of the cells under an inverted microscope yielded similar results for the number of CFU-GMs between lower side populations in the wild type and the induced conditional knockout (*Figure 16*). The striking result was seen between the upper primed cells for the wild type and CK; upper primed cells in the CK tended to be double the amount of those in the wild type (*Figure 17*). What these data mean are still yet to be determined, but it is hypothesized that the lymphoid primed cells may be undergoing a shift in lineage potential toward a myeloid morphology. Since this behavior is observed so early from the stem cells, it lends to another clue as to what may be going on in the overall disease of AML with the involvement of the Nr4a genes.

Future Experiments:

A lack of discussion was provided upon the retroviral infection of the Nr4a3 gene since the experiment was not completed during the time constraints of this project. A replication of this experiment would be needed in order to verify any results obtained and to assure statistical significance.

With the hypothesis that a potential lineage shift may be occurring, it would be beneficial to observe these lymphoid primed cells in particular for both wild type and CK more in depth. It has been suggested that alterations in the chromatin structure are what can shut down certain developmental pathways that lead to commitment to a specific progenitor type[4]. It may be helpful to analyze the chromatin structure of these cells in comparison with wild type cells to observe for anomalies such as translocations. Furthermore, it would also likely be helpful to

perform microarray analysis upon RNA isolated from these cells in order to assess if certain pertinent developmental genes are also turned off in the CK cells. The difficulty in this experiment will arise from the low number of side population cells to begin with and the large amount of mice needed to obtain such a small sample of RNA.

To avoid the potential issue of not being able to isolate enough RNA, an experiment involving RNA-seq should be considered. The benefits of using this procedure over a microarray include the ability to use less of a sample and the receipt of more data. RNA-seq analysis supplies information concerning differential gene expression, post-transcriptional mutations, and the presence of possible gene fusions. Furthermore, this method allows researchers to look at splice variants that could exist for given genes. The drawback to this procedure is that it is very expensive in comparison to microarray analysis. Fortunately, due to continuously improved technologies in the field, it will not be long before experimental procedures such as those involved in RNA-seq will be more affordable for future experiments such as those proposed here.

Conclusion

Observations of a conditional murine knockout for acute myeloid leukemia ($Nr4a3^{-/-}$ $Nr4a1^{flx/flx}$) yielded histochemical data and cell morphological data. CK tissues displayed a large number of blast infiltrates in non-hematopoietic tissues such as the liver, lung, and spleen. These infiltrates were not visible in the wild type tissues. Side population HSC behavior suggested that there may be a shift in lineage potential from lymphoid to myeloid morphology when grown in a MethoCult® Stem Cell Assay for fifteen days. The assay results suggested that the lineage potential shift may also be impacting a variety of developmental pathways that hinder proper lymphoid and/or myeloid progenitor growth. Further research will need to be performed to elucidate the meaning of these results.

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EDUCATION

The Pennsylvania State University, The Behrend College - Erie, Pennsylvania
Schreyer Honor Scholar Graduation: **May 2012**
B.S. in Molecular Biology and Biochemistry, Minor in Mathematics

EMPLOYMENT and RESEARCH

SMART Program Research Participant – Baylor College of Medicine Houston, Texas, May 2011 to July 2011
Mentors: *Dr. Orla Conneely, Ashley Ramirez-Herrick*

Abrogation of orphan nuclear receptor genes NR4A1 and NR4A3 leads to a development of acute myeloid leukemia in a double knockout murine model. Through the use of a conditional model, an attempt to restore proper NR4A1 function through retroviral infection and bone marrow transplants was performed. Hematopoietic stem cell colony assays were utilized to assess lineage potentials for both lymphoid and myeloid progenitors of the wildtype and knockout varieties. An experiment to restore NR4A3 gene function is ongoing. Results from the experiment will be extrapolated to the human form of the disease and used in drug development.

Research Assistant - Penn State University Erie, Pennsylvania, January 2010 to April 2011
Research in conjunction with Rutgers University, Mentor: *Dr. Michael Campbell*

Elucidation and characterization of the biosynthetic pathway of the essential amino acid lysine in the model system *Arabidopsis thaliana* is under investigation. The altered expression of the gene for dihydrodipicolinate synthase (DHDPS) promises to increase lysine expression in cereal grains and corns. Great importance is given to this dietary supplement as it plays a fundamental role in animal growth and development. Laboratory results were clearly organized and documented in professional laboratory reports and regularly communicated to research associates.

ACTIVITIES and HONORS

- Service Chair, Omicron Delta Kappa Junior Senior Honor Society - April 2009 to May 2011
  - Involved group support to various philanthropic and community service efforts including Erie City Mission and Second Harvest Food Bank of Northwest Pennsylvania
  - Co-initiated "No Shave-ember" for Make-A-Wish Foundation®
- Dean's List: Fall 2008, Spring 2009, Spring 2010, Fall 2010, Spring 2011, Fall 2011
- Penn State THON™
- Member, Beta Beta Beta Biological Honor Society, Phi Kappa Phi Honor Society, Gamma Sigma Sigma Service Sorority
- Recipient of various leadership and academic excellence scholarships

LABORATORY SKILLS and PROFICIENCIES

- Extraction of nucleic acids
- Staining and Microscopy
- Quantification of nucleic acids
- QTPCR
- Cell Fractionation
- cDNA synthesis
- Fluorescence Spectroscopies
- Immunohistochemical Staining
- Chromatography
- Electrophoresis
- Aseptic techniques

PUBLICATIONS and PRESENTATIONS

Ramirez-Herrick, AM, Boudreaux, SP, Harrington, EA, Zhang, S, Conneely, OM. **"NR4A Nuclear Receptors Regulate Lineage Bias Potential of Distinct Hematopoietic Stem Cell Subtypes to Prevent AML Development."** (In preparation)

Jones-Held S., Ambrozevicius L., Campbell M., Drumheller B., Harrington E., Leustek T**. "Two *Arabidopsis thaliana* dihydrodipicolinate synthases *DHDPS1* and *DHDPS2* show unequal functional redundancy."** *Plant Journal***.** (In Preparation)

Harrington E. and Drumheller B. **Analysis of Lysine Biosynthesis in Plants Using *Arabidopsis* DHDPS Mutants**, **Spring 2011**, Sigma Xi Conference, The Pennsylvania State University at Behrend Campus. (First Place Prize)