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Fletcher Scholars Award: Progress Report
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Exploiting Darwin to Overcome Drug Resistance in Leukemia

Project Overview and Specific Aims
Rationale: My research is focused on investigating the genetic determinants of therapeutic response, in order to identify prognostic markers for drug resistance and relapse, as well as for therapy-induced second malignancies. This project, among the first at the interface of evolution, germline and somatic genomics, and human disease, is the natural extension of our ongoing research.

It is our hypothesis that there is considerable non-neutral diversity in each patient's cancer that differs between patients who do and do not relapse, and that a critical determinant of clinical outcome is the selection of clones with a survival advantage in the face of chemotherapy. We will test this hypothesis in three specific aims, using high risk *CRLF2*-driven pediatric ALL as our model.

Aim 1: To determine the mutational spectrum in treatment-resistant as compared to treatment-responsive *CRLF2*-driven ALL by whole-exome sequence analysis of patient samples

Aim 2: To establish a humanized mouse model of clonal diversity in *CRLF2*-driven ALL

Aim 3: To use this mouse model to determine the role of therapy on modifying the spectrum of clones in treatment-responsive and treatment-resistant *CRLF2*-driven ALL

As discussed above, evidence that clonal diversity is associated with outcome in ALL is particularly compelling. We predict that by using population genetics methods to study the selective effects of chemotherapy on the emergence of drug resistant clones in a humanized mouse model of relapsed ALL, we will be able to identify the mutations in those clones that drive treatment failure. If so, we may be able to exploit this knowledge to prevent relapse at the time of diagnosis by targeting these mutations. A particular strength of this project is that we will investigate clonal variation within tumors not only in mice before and after chemotherapy, but also in primary samples from the same patients. Thus, we will have a "gold standard" against which we can compare results from our mouse model.

Narrative Account of Progress
Our initial work was dedicated to establishing an animal model for our human ALL xenografts. Our plan was to employ highly immunodeficient (NSG) mice for this work. However, a post-doctoral scholar (and former University of Chicago PhD student) named Mark Sasaki had recently joined my lab. He had previously completed a post-doc at the University of Oregon in developmental biology using zebrafish as a model. His plan was to join my lab in order to learn how to analyze human genomic data in order to then make zebrafish models of human cancers.

Dr Sasaki taught me three important facts about zebrafish: 1) it is easy to introduce foreign cells into the fish on day post fertilization 2 (Dpf 2) by injecting the yolk sac; 2) the fish do not develop a thymus until Dpf 6, suggesting that any foreign cells injected into the fish prior to that date would be likely to be recognized as "self" by the developing zebrafish immune system; and 3) the zebrafish immune system is fully mature by Dpf 21.

These facts led us to hypothesize that zebrafish would be a far superior model for this project than immunodeficient mice. Our rationale was, first, because fish are hardy and can be bred in clutches of up to 600 fish, allowing for tremendous statistical power for our studies, and also allowing us to test many different drug combinations on our human ALL samples, towards the ultimate goal of personalizing therapy by identifying and treating drug resistant clones. This is in contrast to the cost of the NSG mice we planned to use, which are poor breeders, very expensive to maintain, and which, as a consequence, vastly limited the experiments we can do. In addition, we believed that the extreme immunodeficiency required for human leukemias to grow in mice was so artificial that it did not mimic the human case; this was especially true because of literature demonstrating that differing degrees of murine immunodeficiency were associated with the survival of differing human leukemia clones. Thus, we were very concerned that by virtue of our mouse model, we would select against the rare drug resistant clone we hoped to study. In addition, since human patients with leukemia are not immunodeficient, we were concerned that working in immunodeficient mice would lead us to false conclusions that would be overcome using zebrafish to establish long-term xenografts that could be treated after the zebrafish immune system had matured. Finally, we also anticipated that by labeling leukemia cell membranes with a fluorescent dye would provide a simple and quantitative tool to measure the leukemia burden in optically clear zebrafish before and after therapy.

Thus, we decided to establish the first human xenograft model of leukemia in zebrafish. The actual catalyst for this decision was the recent recruitment of Jill de Jong, MD, PhD, a fellow pediatric hematologist/oncologist and zebrafish expert interested in hematopoietic stem cell development. As her interest in normal stem cells and our interest in leukemic stem cells synergized well, we decided to collaborate on developing this model, taking advantage of her lab's knowledge and infrastructure, my lab's genetic and evolutionary expertise, and with my post-doctoral scholars, Dr Sasaki and Eric Hungate, PhD (who obtained his PhD in the Department of Ecology & Evolution with Chung-I Wu, a close collaborator on this project) designing, performing, and interpreting the experiments.

To date, we have successfully demonstrated that we can xenograft human leukemias into zebrafish when they are infused on dpf 2. We have shown that we can inject as few as 100 cells into the yolk sac and that leukemias will grow in >80% of fish. In contrast, when we infuse leukemias into the fish on dpf 15 or dpf 22, when their immune system is either nearly or completely matured, we have never successfully had a human leukemia grow out, suggesting that our hypothesis is correct.

Using this model, we currently have a paper under review at *Nature*, with Dr Weinstock, in which we investigate the influence of a new targeted therapy on clonal selection and treatment

of pediatric ALL. Using zebrafish, we are able to test multiple combination therapies on the cells, as we had hoped.

Dr Hungate and I are also collaborating with Dr Wu on another manuscript currently under review at the *NEJM* exploring clonal diversity in tumors.

Finally, with Dr de Jong, we expect to submit two papers this year, one describing our new zebrafish model for primary xenografts, and the other describing methods we developed for infusing cells into fish older than dpf 3, in whom he yolk sac has disappeared, but younger than dpf 21, in whom intra-peritoneal injection methods have been well established.

Publications since 2012 include:
Cozen, W., Li, D., Best, T., Van den Berg, D.J., Cortessis, V.K., Skol, A.D., Mack, T.M., Glaser, S.L., Gourraud, P.A., Weiss, L.M., Nathwani, B.N., Bhatia, S., Schumacher, F.R., Edlund, C.K., Hwang, A., Strong, L.C., Robison, L.L., Conti, D.V., and **Onel, K**. 2012. A genome-wide meta-analysis of nodular sclerosis Hodgkin lymphoma identifies risk loci at 6p21.32. Blood 119(2): 469-75.

Doçi, C.L., Mankame, T.P., Langerman, A., Ostler, K.R., Kanteti, R., Best, T., **Onel, K.**, Godley, L.A., Salgia, R., and Lingen, M.W. 2012. Characterization of NOL7 Gene Point Mutations, Promoter Methylation, and Protein Expression in Cervical Cancer. Int J Gynecol Pathol 31(1): 15-24.

Onel, K.B., and **Onel, K**. 2012. Tumor Necrosis Inhibitors and Cancer in Juvenile Idiopathic Arthritis: Disentangling the Web. Arthritis and Rheumatism 64(4): 966-69.

Churpek, J.E., Lorenz, R., Nedumgottil, S., **Onel, K**., Olopade, O., Sorrell, A., Owen, C.J., Bertuch, A.A., and Godley, L.A. 2012. Proposal for the Clinical Detection and Management of Patients and their Family Members with Familial Myelodysplastic Syndrome/Acute Leukemia Predisposition Syndromes. Leuk Lymphoma. *In press.*

Elena JW, Travis LB, Simonds NI, Ambrosone CA, Ballard-Barbash R, Bhatia S, Cerhan JR, Hartge P, Heist RS, Kushi LH, Lash TL, Morton LM, **Onel K**, Pierce JP, Robison LL, Rowland JH, Schrag D, Sellers TA, Seminara D, Shu XO, Thomas NE, Ulrich CM, and Freedman AN. Leveraging Epidemiology and Clinical Studies of Cancer Outcomes: Recommendations and Opportunities for Translational Research. JNCI. *In press.*

Lapping-Carr G, Skol AD, and **Onel K**. Genetics, Genomics, and Human Disease. Turkish Journal Science and Technology. *In press.*

Morton LM, Dores GM, Tucker MA, Kim CJ, **Onel K**, Gilbert ES, Fraumeni JF, Curtis RE. Evolving risk of therapy-related acute myeloid leukemia following cancer chemotherapy among adults in the United States, 1975-2008. Blood. *In press.*

With regard to lectures, since *June, 2012*, I have given the following invited talks:

Newcastle University
Newcastle, UK
September 6, 2012

Systems Radiation Biology Meeting
Oxford, UK
October 3, 2012

University of Pennsylvania Cancer Center seminar
Philadelphia, PA
November 6, 2012

Northwestern University Cancer Center seminar series
Chicago, IL
February 21, 2013

The Bone Marrow Niche, Stem Cells, and Leukemia: Impact of Drugs, Chemicals, and the Environment Meeting
New York Academy of Sciences
May 30, 2013

Interlymph Annual meeting
Dijon, France
June 26, 2013

IARC
Lyons, France
June 28, 2013

University of Washington/Fred Hutchinson Cancer Center
Seattle, WA
July 2, 2013

Japanese Society of Hematology annual meeting
Yokohama, Japan
October 3-5, 2013