Introduction to the Revised K23 Application:

I would like to thank the Review Panel for their thoughtful review and the enthusiasm for my revised application which improved dramatically from a merit score of 2.1 to 1.6. The reviewers recognized my outstanding mentoring team, the improvements to my career development plan, and the re-design of the clinical and laboratory endpoints in my research plan which they felt adequately addressed their concerns about the interpretation of the results. Two of the reviewers shared one remaining critique which I will address below. My progress since the initial application was also recognized, and as I have now completed my fellowship training my productivity has increased. I will briefly summarize my accomplishments since submission of the last revision.

Critique by the Review Panel:

Although the reviewers felt that I had been very responsive to their suggestions and had adequately addressed the previous concerns about the trial design for the interpretation of endpoints, there was one remaining critique for my proposal. They expressed concern about the overly ambitious nature of the multiple cohorts (3 proposed) included into the phase 2 testing of the Toll-like receptor 7 agonist in Specific Aim 2. I have enrolled 7 patients onto this trial and have gained some valuable insight from my experiences to date. I agree with the critique and I now fully appreciate the importance of maintaining research focus to insure maximum productivity. Although my interest in exploring these therapies for a variety of malignancies persists, for this revised application I have decided to follow the reviewers’ suggestion and completely focus the TLR-7 agonist trial on a single disease cohort.

The initial proposal was to study 3 cohorts, CLL, ALL and breast cancer. Those cohorts were selected based on the strong supporting pre-clinical and clinical data, and the decision of which cohort to keep was challenging. With the input of my mentoring team I have decided to focus on patients with CLL. This choice was driven mainly by data in the in vitro data generated by my collaborator Dr. Wei Chen who has shown that B-cell CLL cells express TLR-7, the ligation of which induces up-regulation of co-stimulatory molecules and an antigen presenting cell-like phenotype. Furthermore, TLR-7 agonists stimulate apoptosis of patient CLL samples in vitro. Although ALL cells also express these receptors and have similar in vitro response to TLR-7 agonists, the clinical challenges of this patient population led me to focus on CLL. As the reviewers can appreciate, patients with refractory acute leukemia have very poor prognoses. It is less likely that these acutely ill, rapidly progressing refractory acute leukemia patients will respond to an immunologic agent. In addition, my own expectation, which I think is supported in the literature, is that biologic agents are unlikely to be efficacious when used alone especially in acutely ill patients. Furthermore, the acute leukemia cohort presented the added challenge of providing aggressive supportive clinical care which is less complicated in the CLL cohort. Therefore, I have opted to start by testing in CLL, a more indolent disease, where the patients are more likely to complete full treatment cycle without use of other confounding therapies. Since I rely heavily on biologic endpoints from correlative laboratory studies, I think this choice is wise. Lastly, our preliminary experience, although anecdotal, shows “proof of principal” clinical responses in lymphoid hematologic diseases.
The reason I selected CLL over breast cancer is solely based on laboratory data. Although my collaborators have studies in progress with breast cancer cell lines, no data are available yet to know if our pursuit in breast cancer is well founded other than as a better means of activating NK cells. If this were an R21 or R01 application, breast cancer may have been a better choice to stay focused on one disease. However, after much discussion with my mentors, we thought that CLL was the best choice based on solid pre-clinical data, feasibility, and probability of success.

Over the five years of funding, I am confident that progress with clinical and laboratory results will help guide the further directions of my research with the TLR-7 agonist. For example, if laboratory data demonstrate a unique activation mechanism of NK cells, suggesting the potential for synergy, then combining a TLR-7 agonist with the adoptive NK cell therapy in Aim 1 would be the logical next step. As I fully expect that the clinical outcomes will leave room for improvement, I believe it is important to plan for rational combination therapies using a variety of immune-based strategies. Although at face value treating a solid tumor cohort (breast cancer) in Specific Aim 1 and a hematologic disease cohort (CLL) in Specific Aim 2 is somewhat divergent, my mentor group and I think this approach will offer the best opportunities for my career development. My main objective over the next two years is to understand and generate solid evidence for a single disease focus as part of an R21 or R01 application.

**Continued Progress:**

As a new Assistant Professor, my protected time for research is already in place due to the strong commitment to my career development by the Department of Medicine and by my mentors in the Division of Hematology, Oncology and Transplantation. Now that I am free of the clinical and other responsibilities that were part of my fellowship training I have been able to increase my productivity towards my academic goals. Summarized below are some of my accomplishments since my previous application.

1) As part of my career development plan, I have completed the following courses: Biostatistics I, Epidemiologic Methods I, Fundamentals of Clinical Research, Management of Clinical Research and a seminar designed for K12 and K23 awardees, Topics in Clinical Research (14 credits). This semester I am taking Biostatistics II, Epidemiologic Methods II and the Clinical Research Seminar: Career Development in Clinical Research (9 credits).

2) I have enrolled 3 patients onto the Allogeneic NK Cell protocol in Specific Aim 1, completing 25% of the accrual, and I have enrolled 7 patients onto the TLR-7 agonist trial.

3) I have a new first author publication which was a review entitled “Adoptive Therapy with T-Cells/NK Cells” published in the *Biology of Blood and Marrow Transplantation* (13:33-42, 2007), which is included in the application appendices.
4) I am first author on another paper accepted to Blood (pending minor invited revisions) entitled “A Subpopulation of Human Peripheral Blood NK cells Lacking Inhibitory Receptors for Self MHC is Developmentally Immature”. I expect formal acceptance and publication shortly after this application. The manuscript contains novel information regarding subsets of NK cells which lack receptors recognizing “self” MHC molecules typically felt to be important for mechanisms of tolerance. These findings are critically important in understanding immune development in patients with cancer and after transplantation.

5) I am first author (with my mentor Dr. Miller) on a book chapter NK cell therapy which has been submitted to the publisher.

6) I am second author on a publication accepted to Blood entitled “Missing KIR-ligands is associated with less relapse and increased graft versus host disease (GVHD) following unrelated donor allogeneic HCT” (in press).

7) I am a coauthor on a publication entitled “First in human phase I trial of a novel systemic TLR7 agonist to activate innate immune responses in patients with advanced cancer” which was recently submitted to the Journal of Clinical Oncology.

8) I gave oral presentations at an the International Society of Experimental Hematology in September, 2006, at an international NK cell meeting in Dusseldorf in October 2006, and at the American Society of Blood and Marrow Transplant Meeting in February 2007 and a poster presentation at the American Society of Hematology in December, 2006. I received a competitive new-investigator travel award for my ASBMT presentation.

9) I have continued to make progress in defining my academic niche within the infrastructure of the Cancer Center. Working with my biostatistics and clinical mentors, I have developed a laboratory outcomes database to integrate with the already established Clinical Outcomes Database. This accomplishment will be critically important for future success in my career. Although data management for the small trials proposed here is feasible, manual analysis of the data is inefficient and error prone. Investment of my time in this effort is clearly based on future planning and recognizing the importance of such resources. It has also been an outstanding educational experience to truly learn about the complexities of data analysis which cannot be learned in the classroom alone.

In summary, I believe that my continued progress has been documented above with concrete benchmarks of accomplishment. The tighter research focus in the current proposal will ensure that I am able to collect complete and interpretable data in a short time, to allow me to develop my subsequent research questions and to direct my next investigations. Overall, the career development and research plans presented here will allow me to become an independent and funded clinical research investigator.