2015-2016 Novel Clinical and Translational Methods (NCTM) Pilot Program Application

Name(s): Christopher C. Porter, MD

Department(s) and School/College: Pediatrics, School of Medicine

Project Title: Development of novel method for targeted delivery of calcineurin inhibition

Applying for:
• Phase I (Identify Novel Methodological Development Need)
• Phase II (Novel Method Development Plan)

This application qualifies as:
• Underserved and Minority Investigator
• Female Principal Investigator
Objective:

We have found that leukemia cells are dependent upon the phosphatase calcineurin to evade immune destruction. While calcineurin inhibitors such as cyclosporine and tacrolimus have been used clinically for decades, systemic inhibition of calcineurin is ineffective in treating leukemia because of the potent immune suppressive activity of these drugs. Thus, our objective is to deliver calcineurin inhibition specifically to leukemia cells in vivo, while sparing immune cells. Targeted delivery of calcineurin inhibition would allow us to determine whether pharmacologic inhibition of calcineurin in leukemia cells promotes an immune response to leukemia cells leading to remission of the disease. These findings would have significant translational implications for the treatment of leukemia and perhaps others cancers, and could lead to the development of novel drugs for leukemia.

Background and rationale:

Evasion of the immune system is considered one of the defining features of cancer. However, the mechanisms that cancer cells engage for immune evasion in carcinogenesis, particularly for leukemia, are incompletely understood. While great achievements have recently been made in programming immune cells to target cancer cells, to more efficiently and fully exploit the potency of the immune system in preventing and treating cancer, a greater understanding of immune evasion mechanisms is urgently needed.

Our data suggest a novel mechanism of immune evasion during leukemogenesis. We have found that calcineurin deficient acute lymphoblastic leukemia (ALL) cells engraft in the bone marrow (BM) of un-irradiated, syngeneic, immune-competent recipient mice, but are rapidly suppressed to the point of being undetectable (Figure 1A). However, transplantation into immune compromised recipients abrogates this phenomenon (Figure 1B), strongly implicating immune mediated suppression. Importantly, treatment of recipient mice with the calcineurin inhibitor cyclosporine, does not prolong survival of recipients of the control leukemia, and actually shortens the survival of recipients of leukemia cells without calcineurin (Figure 1C), probably due to inhibition of T cells by cyclosporine. Ongoing studies are designed to better understand the molecular and cellular processes involved in immune-mediated elimination of leukemia cells, but in order to therapeutically exploit leukemia cells’ dependence upon calcineurin, an inhibitor will have to be delivered without affecting the immune system.

Our long-term goal is to develop a greater understanding of how leukemia cells evade immune-mediated elimination and exploit this knowledge for the treatment of leukemia. The overall objective of the current proposal is to deliver calcineurin inhibition specifically to leukemia cells in vivo, while sparing immune cells. Our hypothesis is that specifically inhibiting calcineurin in leukemia cells, and not in immune cells, will promote immune-mediated destruction of leukemia cells. While cyclosporine and tacrolimus are FDA approved drugs, they are potently immunosuppressive. To our knowledge, there are no methods for targeted delivery of calcineurin inhibition.
Figure 1. Leukemia-cell calcineurin promotes immune evasion during leukemogenesis
A. Un-irradiated, wild-type (WT) C57Bl/6, or immune compromised (Tcra−/−) mice were injected with 5x10⁵ luciferase expressing BCR-ABL1+/Arf− leukemia cells with either control shRNA (shNS) or shRNA against Ppp3r1 (shCN-351), which encodes the essential subunit B of calcineurin (CnB). Over 90% knockdown of CnB in a clonal population was confirmed by western blot (not shown). Recipient mice were imaged periodically via IVIS, 5 minutes after injection of luciferin. While shCN leukemia progresses and then regresses in immune competent WT recipients, it progresses unabated in immune compromised recipients. B, C. Un-irradiated, WT mice were injected with leukemia as in Figure 1. Half of the recipients (shNS or shCn) were treated with cyclosporine (CsA) 25mg/kg/d (or vehicle) by oral gavage, beginning on day 3 (n=5/group). In recipients of shNS leukemia, CsA modestly but significantly enhances leukemia progression as compared to vehicle treated recipients (P=0.04 at day 10). CsA treatment led to maintenance of disease burden in some recipients of shCn leukemia, while none of the vehicle treated mice had detectable leukemia by day 10 (P=0.006). All of the CsA treated recipients of shCn leukemia had progression of leukemia, while 3 of the vehicle treated shCn recipients remained in apparent remission to day 150. A second, identical experiment corroborated these findings. The difference in leukemia progression correlates with shorter survival in the CsA treated recipients of shCn compared to vehicle.