Progress Report

Development of a Pharmacological Activator of STAT1 for the Targeted Therapy of Chronic Lymphocytic Leukemia

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Summary of proposal. The focus of this proposal is to utilize our increased understanding of the molecular abnormalities that underlie CLL to develop rational molecular therapeutic approaches for the treatment of this disease. In previous research, we had determined that abnormalities in the phosphorylation of the transcription factor STAT1 on a unique serine residue was a ubiquitous feature of CLL. Furthermore, biological therapies used to treat CLL result in the activation of STAT1, and clinical response correlates with the activation of this protein. Based on these findings, and confirmatory in vitro work, we initiated experiments to identify potential drugs that could activate STAT1 and enhance its function. Through this approach, we identified a compound, 2-(1,8-Naphthyridin-2-yl)phenol (2-NP), that can enhance STAT1 function in CLL cells. We thus proposed to determine the effects of 2-NP on the biology of CLL cells, alone and in combination with other therapies for CLL, in cell culture systems and animal models.

Research progress. Using samples from untreated patients with CLL cultured in vitro, we have shown that 2-NP increases STAT1 target gene expression alone, and enhances the activation of these genes when used in conjunction with the biological agent bryostatin 1. Furthermore, 2-NP increases the degree of differentiation of CLL cells when used in conjunction with bryostatin 1, as assessed by CD22 expression. When CLL cells are treated with the combination of 2-NP and fludarabine or the monoclonal antibody rituximab, enhanced cell killing can be seen when assessed at 72 hours. Notably, peripheral blood mononuclear cells from normal donors are unaffected by addition of 2-NP to any of these therapies, reflecting the fact that abnormal STAT1 phosphorylation is restricted to CLL cells. These results strongly support the hypothesis that pharmacological activation of STAT1 may be an extremely effective therapy for CLL.

The second aim of the proposal focused on the use of 2-NP in a transgenic mouse model of CLL. In collaboration with several of our colleagues at Dana-Farber, we have been developing a system to propagate human CLL cells in immunodeficient mice. As this system seems more beneficial for modeling human CLL, we have deferred initiation of this aim pending validation of this model. However, we are fully committed to proceeding with the pre-clinical studies necessary to introduce STAT1-directed molecular therapy into clinical trials for patients with CLL.

Future directions. We are taking a three-pronged approach to following up on this research. First, as noted, we are planning on initiating studies of 2-NP in an animal model of CLL, to determine the potential of this approach for treating CLL patients. Second, to accelerate the introduction of STAT1-directed therapy of CLL, we are identifying drugs that are already known to be safe in humans that enhance STAT1 function, and which can be introduced into clinical trials in patients with CLL in the near term. An integral part of such a study would be recovery of CLL cells from treated patients to assess and optimize the activation of STAT1 for therapeutic purposes. This pharmacodynamic component of these studies would be essential to optimizing this strategy for clinical use. Finally, increasing evidence suggests that STAT1 and the highly related transcription factor

STAT3 may play reciprocal roles in gene activation and the biology of CLL cells. We have identified several drugs that are effective and specific inhibitors of STAT3, and we are evaluating their potential as a component of molecular therapy for patients with CLL.

We are extremely grateful for the generous support of the CLL Foundation. As noted above, it has been instrumental in moving forward our translational research whose goal is to introduce new molecular therapies for CLL that will be more effective and less toxic. We believe several publications and presentations will emanate from this work, and we will, of course, acknowledge support from the CLL Foundation in those. I would also welcome the opportunity to assist the CLL Foundation in any of its activities that will enhance the therapy of this common though incurable disease.