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Overcoming stroma-mediated treatment resistance in chronic lymphocytic leukemia through BCL-2 inhibition

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Abstract

Despite recent advances in the treatment of chronic lymphocytic leukemia (CLL) with chemoimmunotherapy, many CLL patients are older or frail and cannot tolerate aggressive chemotherapy. Even those who can inevitably develop resistance due to factors such as deletion of pro-apoptotic factors like *TP53* or the support of pro-survival signals from the stromal microenvironment. Using BH3 profiling, we found that CLL cells co-cultured with stroma are less primed to undergo apoptosis in response to BCL-2 inhibition. Currently, several approaches to BCL-2 inhibition with well-tolerated oral agents are in development in the clinic. Dosing of Navitoclax (ABT-263) was complicated by thrombocytopenia due to BCL-XL inhibition, but the BCL-2 specific inhibitor ABT-199 (GDC-0199) should avoid this issue, and may overcome stroma-mediated resistance to apoptosis. We are developing BH3 profiling as a biomarker to predict response to novel therapies such as ABT-199, and to identify resistance mechanisms to new agents being studied in CLL.

Keywords

Chronic lymphocytic leukemia; apoptosis; B cell lymphoma/leukemia 2 (BCL-2); microenvironment

INTRODUCTION

The treatment of patients with relapsed refractory chronic lymphocytic leukemia (CLL), particularly those with fludarabine-refractory disease or high risk markers such as del(17p), remains a major challenge. There is no clear standard of care for these patients, and with currently approved therapies the median overall survival is generally less than 6 months[1]. The efficacy of most chemotherapy is dependent on the reliable transmission of pro-death signals that eventually result in cell death through the intrinsic pathway of apoptosis at the level of the mitochondrion. One of the fundamental problems with chemotherapy in refractory CLL is that the upstream mediators of pro-death signaling are often mutated or deleted, with the best known example, del(17p) and the resulting deletion of *TP53*, occurring in about 20% of relapsed patients and up to 40% to 50% of fludarabine-refractory

CLL patients[2]. Another important source of resistance to chemotherapy is that CLL cells in lymph nodes and bone marrow are bathed in a protective microenvironment that includes a variety of pro-survival signals. Recently, agents that target the B cell receptor (BCR) have shown promising results in CLL[3], and it is thought that much of their activity may result from mobilization of CLL cells from sanctuary stromal sites into the peripheral blood, where the cells are more sensitive to death signaling.

PRECLINICAL STUDIES

Our group has been interested in studying the effects of the microenvironment on the propensity for CLL cells to undergo apoptosis through the intrinsic pathway involving the BCL-2 family of proteins. To accomplish this, we have used well-established models of the CLL microenvironment such as *in vitro* co-culture of CLL cells with stromal cells including nurse-like cells (NLCs) or cell lines such as StromaNKTert, as previously described[4]. The main measure of the proximity of CLL cells to the threshold of apoptosis is what we refer to as “priming”. Cells that are highly primed lie close to this apoptotic threshold and are easily killed with a variety of treatments. Unprimed cells are far from the apoptotic threshold, and are often resistant to multiple therapies. To quantify the level of priming, we utilize a technique developed by our group called BH3 profiling[5]. This functional assay involves incubating gently permeabilized CLL cells with different BH3-only peptides that enter into the cells and interact with the native BCL-2 family proteins at the mitochondria. Cells primed for apoptosis will undergo mitochondrial outer membrane permeabilization (MOMP) and mitochondrial depolarization in response to BH3-only peptides more readily than unprimed cells.

We recently demonstrated that the level of priming is clinically relevant by showing that patients with previously untreated CLL who had a clinical response to front line therapy had higher levels of priming than non-responders[6]. It had previously been observed that contact with stroma made CLL cells more resistant to apoptosis induced by BCL-2 inhibition *in vitro*[7]. Therefore, we tested whether co-culturing CLL cells with stroma decreases CLL cell priming, and we found that it did. To gain insight into whether this *in vitro* observation was relevant to the *in vivo* situation, we also utilized matched patient samples to show that CLL cells derived from the bone marrow are significantly less primed than those from the peripheral blood.

Given the critical role played by the BCR in transmitting stroma-mediated pro-survival signals, we also examined the effect of anti-IgM-mediated BCR activation on priming, and found that priming decreased significantly. Building on this finding, we also showed that the delta-isoform phosphoinositide-3-kinase inhibitor GS1101 (formerly CAL-101) was able to partially overcome stroma-mediated resistance *in vitro* by raising the level of CLL cell apoptotic priming. This finding helped explain our additional observation that the combination of GS1101 with the BH3-mimetic ABT-737 led to particularly potent CLL cell killing even in the presence of stroma.

CLINICAL STUDIES

To overcome stroma-mediated treatment resistance in CLL, a novel therapy would need to bypass dysfunctional upstream cell signaling such as absent or mutated *TP53*. It would also need to be tolerated at a dose high enough to overcome pro-survival signals in sanctuary sites like the bone marrow. Over the last several years, a number of different pharmacologic approaches have been taken to target the BCL-2 family in CLL. Of these, the three agents that are the furthest along clinically are obatoclax, oblimersen, and ABT-263/ABT-199.

Obatoclax (GX-15-070, Teva Pharmaceuticals, Petah Ikva, Israel), is a pan-BCL-2 family member inhibitor, with modest affinity for BCL-2, BCL-XL, and MCL-1 *in vitro*[8]. The effect of the drug *in vivo* is less clear, and the fact that it does not cause significant thrombocytopenia suggests that it may not effectively inhibit BCL-XL, which is known to be a key pro-survival protein for platelets. Patients with relapsed CLL treated on a study of fludarabine/rituximab plus obatoclax had a reasonably good time to progression of 20 months[9], but drug availability issues have recently hampered further exploration of obatoclax in CLL.

Oblimersen (Genasense; Genta, Berkeley Heights, NJ) is a single-stranded, phosphorothioated 18-mer DNA molecule with a complementary antisense sequence to the mRNA of BCL-2[10]. Although oblimersen reduces BCL-2 protein levels in cell lines, it is unclear how effectively it does this *in vivo*. The drug did demonstrate a 50% reduction in the risk of death in fludarabine-sensitive patients when used in combination with fludarabine/cyclophosphamide (FC) compared to patients treated with FC-alone in a randomized, phase 3 study[11]. However, the future of oblimersen in the treatment of CLL remains uncertain at this time.

Currently, the most promising approach to targeting BCL-2 in CLL is through the BH3-mimetic drugs Navitoclax (ABT-263) and ABT-199 (GDC-0199) (Abbott Laboratories, Abbott Park, IL and Genentech, South San Francisco, CA), which are structurally similar to the potent *in vitro* BH3-mimetic ABT-737. Like ABT-737, Navitoclax also targets BCL-XL and BCL-W. Navitoclax has demonstrated significant activity in the clinic. In the initial studies, efficacy was greatest in CLL patients, with one study showing 50% (8/16) of CLL patients achieving a partial remission (PR), and 81% (13/16) showing at least some reduction in tumor size [12]. In a CLL-specific phase I study with expansion, PR was achieved in 31% (9/29) of patients, with an additional 62% (18/29) of patients at least maintaining stable disease, resulting in a median progression-free survival of 25 months[13]. This study was followed by a phase 2 study of Navitoclax in 31 patients with relapsed refractory CLL, which at an interim analysis reported PR in 38% (10/26) of patients, including 2 patients with del(17p)[14].

Thrombocytopenia due to on-target BCL-XL inhibition was the major dose-limiting toxicity of Navitoclax, and was dose-related, with 27% of patients on the phase 2 study demonstrating grade 3/4 thrombocytopenia. Because patients with relapsed CLL often have significant baseline thrombocytopenia due to prior therapies or CLL bone marrow infiltration, the drug-induced thrombocytopenia from Navitoclax is a serious limitation of

this agent, potentially limiting the ability to push the drug up to high enough doses to overcome stroma-mediated resistance to apoptosis. For example, several patients on the phase 2 study of Navitoclax had a significant amount of residual bone marrow CLL infiltration, despite having low circulating lymphocyte counts. Furthermore, the fact that Navitoclax is continuously dosed makes it challenging to manage severe thrombocytopenia, since transfused platelets are subject to the same BCL-XL inhibition as native platelets, thereby reducing their circulating half-life as well.

To realize the potential benefits of BCL-2 inhibition without the detrimental effect of BCL-XL-mediated thrombocytopenia, a successor molecule, ABT-199 (GDC-0199), is now being developed. ABT-199 is a small molecule, orally-bioavailable, BCL-2 specific inhibitor with a greater than 100-fold selectivity for BCL-2 over BCL-XL. It is therefore not expected to cause thrombocytopenia. A large first-in-human, phase 1, multicenter, international dose-escalation trial for patients with CLL and non-Hodgkin lymphoma opened in June 2011 and is ongoing, with a goal accrual of approximately 118 patients. The primary objectives of the study are to assess safety and pharmacokinetics and to determine the maximum tolerated dose of ABT-199. Secondary objectives include efficacy assessments as well as analysis of biomarkers. It is hoped that without the dose-limiting thrombocytopenia seen with Navitoclax, the dose of ABT-199 will be able to be pushed high enough to overcome stroma-mediated treatment resistance in sanctuary sites such as the bone marrow. Building on this initial monotherapy study, a number of combination studies are also now underway, including separate phase 1 studies of ABT-199 plus rituximab, ABT-199 plus obinutuzumab (GA-101), and ABT-199 plus bendamustine/rituximab.

BIOMARKER STUDIES

Though BCL-2 inhibition holds great promise as a therapeutic target, not all patients will respond, and many will likely develop resistance mechanisms that lead to eventual disease progression. For example, a compensatory upregulation of other anti-apoptotic proteins such as MCL-1 could decrease the efficacy of a BCL-2 specific inhibitor. Therefore, the development of robust predictive biomarkers for BCL-2-targeted therapies is critical. Correlative data from the phase 1 study of Navitoclax in CLL showed that low MCL-1 expression and high BIM:MCL-1 or BIM:BCL-2 ratios in CLL cells are associated with response[13]. If further validated, measuring individual protein expression levels would be a straightforward way to predict response to BH3-mimetics.

However, given the complex biology of the BCL-2 family, the expression level of individual proteins alone may not optimally reflect the biology of CLL *in vivo*. For example, BCL-2 family proteins are subject to a variety of post-translational modifications and protein-protein interactions that can have a significant influence on the fate of a cell. As BH3 profiling is a physiologic assay that probes the interactions of over a dozen pro- and anti-apoptotic BCL-2 family members, we are currently exploring whether it may serve as an even more robust predictor of response to targeted therapies. Baseline peripheral blood and bone marrow samples from patients on studies such as the ABT-199 monotherapy trial are being collected, and BH3 profiling is performed to determine whether the level of apoptotic priming is associated with response to treatment. We will also profile samples at time of

relapse to determine whether, for example, increased dependence on MCL-1 is an important resistance mechanism in CLL patients treated with ABT-199.

DISCUSSION

After a decade in the 2000s where much progress in CLL therapy was made with the establishment of chemoimmunotherapy as a standard of care for younger, fitter patients, the 2010s have started with a flurry of activity with oral agents targeting the molecular abnormalities thought to be fundamental to the pathophysiology of CLL. Though BCR antagonists have advanced the furthest in clinical trials, BCL-2 inhibitors such as ABT-199 are now moving forward quickly, and are also likely to play an important role in the future therapy of CLL. Since these oral agents are better tolerated than chemotherapy, they open the possibility for dramatic improvement in the treatment options available for all CLL patients, including those who are older and less fit than those traditionally included in clinical trials. Correlative laboratory studies in these trials will be critical to determine mechanisms of resistance to these agents, and to ascertain whether predictive biomarkers such as BH3 profiling can eventually be used to prospectively guide therapy of patients with CLL.

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