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Opportunities for targeting gene regulatory factors in B-cell acute lymphoblastic leukemia

Christopher J Ott^{*,1}

¹Dana-Farber Cancer Institute, Harvard Medical School, 450 Brookline Avenue, Boston, MA 02215, USA

* Author for correspondence: christopher.ott@dfci.harvard.edu

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First draft submitted: 12 June 2017; Accepted for publication: 7 July 2017; Published online: 20 November 2017

Keywords: bromodomain • HDAC • MLL

Modern chemotherapy regimens have had a profound impact on the prognosis of individuals diagnosed with B-cell acute lymphoblastic leukemia (B-ALL), especially children [1]. However these treatments achieve substantially poorer survival rates in adults, and are associated with significant short- and long-term toxicities. An advanced understanding of the genetic complexity that characterizes B-ALL allows for more precise patient stratification, and reveals disease subtypes that are at a high-risk for treatment relapse [2]. This genetic profiling has also revealed opportunities for the development of targeted therapies. For example, recurrent *BCR-ABL1* translocations define a unique subtype of ALL found in 2–4% of pediatric and at least 25% of adult cases. The activity of this oncogene can be effectively inhibited with the tyrosine kinase inhibitor imatinib, with promising clinical results [3]. Intriguingly, many ALL oncogenes include gene fusions and translocations of transcription regulators and other chromatin associated factors (e.g., *ETV6-RUNX1*, *TCF3-PBX1*, *MYC* or *MLL* rearrangements), revealing the disease to be primarily one of disrupted gene expression control. The mechanistic aspects of these oncogenes are incompletely understood, yet remain an active area of research. These factors can unfortunately also often be viewed as intractable for therapeutics development. Yet much recent progress has been made in targeting gene regulatory complexes with small molecule pharmaceutical agents [4]. Additionally, promising preclinical and early clinical data in B-ALL is emerging with drugs that target these factors. Here are highlighted several of these new drug classes, including inhibitors of bromodomains, histone deacetylases (HDAC) and MLL fusion complexes.

Bromodomain inhibitors in B-ALL

Bromodomains are specialized protein modules that specifically recognize (or ‘read’) lysine residues that have been post-translationally acetylated by acetyltransferase enzymes. These domains are found in 46 proteins of the human proteome, and are often found in factors that associate with chromatin and regulate gene expression [5]. While pharmacologic inhibition of protein–protein interactions has historically been a challenge, advances in drug development technology now allow for discovery and rational design of pharmaceutical agents that can efficiently block select interactions in cells. This is convincingly evidenced by the development of venetoclax and similar compounds in use for hematologic malignancies. These compounds block binding of the pro-apoptotic BCL2 factor with the anti-apoptotic BH3 family factors, leading to apoptosis induction in treated cells [6]. High-throughput screening and medicinal chemistry efforts have realized several classes of compounds that efficiently displace bromodomain-containing factors from acetylated proteins. The most significant progress has been made with drugs that specifically inhibit the bromodomain and extra-terminal domain-containing (BET) family of proteins that associate with acetylated chromatin to regulate gene expression [7]. These compounds can have relatively selective effects on gene expression through perturbation of unique gene enhancer elements [8]. In B-ALL, BET inhibition results in potent decreases in expression of the *MYC* and *IL7R* oncogenes [9,10]. JQ1, a prototypical

BET inhibitor, displaces the BET family member BRD4 from gene regulatory elements of these genes, leading to apoptosis in B-ALL cell lines and primary patient-derived cells. Daily JQ1 treatment in patient-derived xenograft murine models of B-ALL leads to significant decreases in tumor burden and increased overall survival. A number of BET inhibitors, including derivatives of JQ1, have entered early clinical trials in hematologic malignancies including ALL, with some observed responses (NCT01713582 [11]).

While inhibitor development for the BET family of bromodomain-containing proteins is more advanced, other bromodomain factors may also be worth targeting in B-ALL. For example, the chromatin factor TRIM33 is a unique dependency in B-ALL and other B-cell types [12]. TRIM33 acts at least in part by binding an enhancer in proximity to the pro-apoptotic gene *Bim*, repressing its expression. Although current bromodomain inhibitors do not effectively target TRIM33, we may hypothesize that development of a selective TRIM33 bromodomain drug may effectively derepress *Bim* expression leading to apoptosis induction in B-cell types.

HDAC inhibitors in B-ALL

The HDAC family is made up of 11 enzymes that include several factors known to modulate chromatin structures in the nucleus. Chemical inhibitors that broadly and relatively nonselectively target the HDAC family – including romidepsin, vorinostat, belinostat and panobinostat – have been approved for use clinically for the treatment of cutaneous T-cell lymphoma or multiple myeloma [13]. Thus, many efforts are ongoing to determine the promise of these compounds across other hematologic malignancies. The activity of some nonselective HDAC inhibitors have been profiled in preclinical models of B-ALL, with bulk hyperacetylation of chromatin and concomitant antiproliferative and pro-apoptotic effects observed [14].

Recently, more selective compounds have become available for study, including compounds that specifically target HDAC1 and HDAC2, members of the class I family of HDACs that are known components of repressive gene regulatory complexes. A recent study profiled HDAC1/2 selective compounds in a panel of B-ALL cell lines, showing roughly equivalent sensitivity as is observed with nonselective HDAC inhibitors [15]. Selective HDAC1/2 inhibitors induce apoptosis and DNA damage response *in vitro*, and can be dosed in xenograft models of B-ALL leading to tumor reduction. Intriguingly, this effect seems to be unique to B-ALL as the activity of these inhibitors is reduced in mature B-cell lymphoma and plasma cell-derived cell lines. Although selective HDAC inhibitors have yet to be clinically approved for use in cancer, several inhibitors with varying selectivity profiles are currently undergoing clinical investigation (NCT01132573, NCT00462605, NCT01321346, among others).

Targeting MLL fusions

Roughly 5–9% of ALLs are characterized by reciprocal translocations of the *MLL* gene (at chromosome 11q23), leading to oncogenic MLL fusion proteins that confer a poor prognosis [2]. MLL is a chromatin-associated factor, and the fusion oncogenes are thought to lead to aberrant association with the genome leading to disrupted gene expression, particularly through upregulation of class I *HOX* genes [16]. Two strategies have recently emerged to directly target MLL-fusion activity. First, MLL-fusions recruit the DOT1L methyltransferase to chromatin, resulting in ectopic methylation of histones in actively transcribed genes including *HOXA9* [17]. This has provided a therapeutic rationale for the pharmacologic inhibition of DOT1L methyltransferase activity, and a series of potent, selective inhibitors have been developed [18]. Pharmacologic DOT1L inhibition leads to decreased MLL-fusion leukemia cell proliferation in cell culture studies and murine xenografts with continuous dosing. DOT1L inhibitors have progressed into early phase clinical studies in children and adults with relapsed/refractory MLL-rearranged leukemia, with some reported transient reduction in pharmacodynamic markers and leukemia blasts (NCT01684150, NCT02141828 [19]). Further clinical testing will be required to determine whether DOT1L inhibition may be effective in this patient population.

Similar to its recruitment of the DOT1L factor, the activity of MLL fusions can be dramatically enhanced by macromolecular complex formation involving other factors. Inhibitors have therefore been developed to block the interactions of MLL with co-complex proteins to antagonize oncogene activity. Recently, small molecules have been designed to inhibit the interaction of MLL with menin, which leads to disruption of MLL-fusion protein activity [20]. These compounds selectively kill leukemia cells that harbor MLL fusion proteins, and are active in mouse models of the disease. As clinical studies for this class of inhibitor are just now initiating, the promise of targeting MLL fusions through this mechanism is yet to be determined.

Thus, while much work remains, there is intriguing experimental evidence to suggest targeting gene regulatory factors with pharmaceutical agents could be an effective therapeutic strategy for B-ALL. Ongoing and future

preclinical and clinical studies will be required to determine the extent of single-agent activity and optimal combination regimens, which will likely be inferred from results gleaned from studies in other malignancies. It is anticipated that through these efforts, more refined treatments for B-ALL will emerge.

Financial and competing interests disclosure

In collaboration with his colleagues, CJ Ott is an inventor on a patent filed by his institution describing the use of bromodomain inhibitors in therapy-refractory ALL. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. CJ Ott gratefully acknowledges funding from a Pathway to Independence Award from NIH/NCI (K99CA190861).

No writing assistance was utilized in the production of this manuscript.

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