



Guest editorial

IL3 receptor-dependent therapeutic targeting of BCR-ABL-driven lymphoid leukemias

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Despite the development of intensive combination chemotherapy, many leukemia patients still succumb to drug-resistant leukemia. In contrast to the success of ‘targeted’ tyrosine kinase inhibitor (TKI) therapy in BCR-ABL-driven chronic myelogenous leukemia (CML) [particularly in chronic phase (CP)], BCR-ABL-driven acute lymphoblastic leukemias (including ‘*de novo*’ Ph+ ALLs, and CML patients in lymphoid blast-crisis [LBC]) invariably relapse while on single-agent tyrosine kinase inhibitor therapy [1]. In these TKI-resistant leukemias, a number of well-characterized drug-resistant BCR-ABL Kinase Domain (KD) mutations frequently emerge in the dominant drug-resistant leukemic clone(s) [2]. While tyrosine kinase inhibitors can be safely added to intensive combination chemotherapy regimens and improve outcome [3], BCR-ABL-driven lymphoid leukemias still remain one of the ‘highest-risk’ leukemias, especially in adult populations.

In this issue of Leukemia Research, Kim et al. [4] present evidence that targeting the Interleukin-3 (IL3) receptor within BCR-ABL-driven lymphoid leukemias represents a viable therapeutic strategy worthy of further investigation. The IL3 (or IL-3) cytokine functions by binding the hetero-dimeric IL3 cell surface receptor complex comprised of the cytokine-specific IL3RA (also referred to as IL-3R α , CD123) and the common CSF2RB subunit (commonly referred to as IL3R β or CD131, a component of receptor complexes for the IL-5 and GM-CSF cytokines). IL3 binding to this hetero-dimeric complex activates a JAK2-dependent signaling cascade, culminating in a STAT5-dependent transcriptional program [5]. Recognition of the frequent and elevated cell surface expression of IL3 receptor subunits in primary leukemic myeloblasts, including leukemic stem cells (*see below*), and IL3’s growth-stimulatory effects on patient-derived myeloblasts (reviewed in Ref. [6]) has prompted the development of IL-3-targeted therapeutics. The Frankel and Hogge research groups have developed [7] and pre-clinically validated [8,9] the utility of the diphtheria toxin-IL3 fusion (DT-IL3) protein DT₃₈₈IL3 that selectively binds to the extra-cellular IL3 receptor complex and eliminates IL3 receptor-expressing leukemic

myeloblasts. Mechanistic studies implicate toxin-dependent, ADP-ribosylation of Elongation Factor 2 (EF2), inhibition of protein synthesis, and subsequent induction of both apoptotic and non-apoptotic cell death as key biological effects of cytokine-toxin fusion proteins [10,11]. Phase I/II clinical trials of GMP-produced DT₃₈₈IL3 in adult patients with relapsed/refractory/poor-risk acute myeloid leukemia or myelodysplasia were initiated in 2006 [12] and remain open to accrual [ClinicalTrials.gov Identifier: NCT00397579]. To date, denileukin diftotox (DAB389-IL2; ONTAK) an IL2-DT fusion protein that selectively targets IL2 expressing cells, remains the only FDA-approved immunotoxin. Initially approved for treatment of patients with cutaneous T-cell lymphoma (CTCL) in 1999, denileukin diftotox has subsequently demonstrated significant efficacy in several B- and T-cell hematological malignancies [10].

Extending prior analyses of IL3 receptor expression on leukemic myeloblasts and on CML-derived lymphoblasts and myeloblasts, Kim et al. [4] characterized significant IL3RA and relatively lesser CSF2RB cell surface expression in primary lymphoblasts recovered from Ph+ ALL and CML-LBC patients. Most importantly, *in vitro* colony-forming-cell (CFC) assays revealed a significantly greater sensitivity of leukemic lymphoblasts than normal hematopoietic progenitors to imatinib (the first generation TKI), dasatinib (a second-generation TKI) and the DT₃₈₈IL3 toxin. These authors also demonstrated synergistic growth inhibitory activity between exposure to either of these TKIs and the DT₃₈₈IL3 toxin, and a clear positive relationship between IL3RA expression assayed by flow cytometry and *in vitro* DT₃₈₈IL3 toxin sensitivity. While actively cycling cells were relatively more sensitive than the quiescent sub-population, synergy between agents was again demonstrated in both sub-populations. Finally, Kim et al. [4] demonstrated similar findings in more proliferative lymphoid progenitors in long-term suspension culture-derived CFC assays.

Might development and utilization of DT₃₈₈IL3 for ‘high-risk’ lymphoid leukemias, such as Ph+ ALL, be optimally achieved? In view of the demonstrated selectivity of DT₃₈₈IL3 toward IL3 receptor-expressing cells [7], selection for treatment of leukemic lymphoblasts which are positive for IL-3 receptor would be a logical approach [12]. As observed in this current study, IL3RA (CD123) expression is typically observed in normal myeloid progenitors but not in normal lymphoid progenitor cells, while virtually all B-lineage lymphoblastic leukemia cases aberrantly expressed this receptor [13–15] at levels often exceeding those in AML [13]. Ultimately, therapeutic targeting of the IL3 receptor in ALL may be most effective when used in combination with conventional chemotherapeutic drugs, particularly in view of differing mechanisms of action [10,11].

The stability and heterogeneity of expression of receptor components for IL3 during the course of conventional and DT₃₈₈IL3-inclusive therapy also warrants further consideration. Encouragingly, IL3RA was stably expressed in residual lymphoid leukemic blasts during the course on induction chemotherapy [15], but whether IL3 receptor expression is maintained or enriched in the ALL leukemic-initiating cell (LIC) sub-population, as observed in AML cases [16], will ideally be investigated in a prospective manner. LICs, alternatively referred to as leukemia stem cells (LSCs) represent those leukemic cells resident within the bulk tumor population that possess self-renewal capacity (hence the reference to 'stem cells'), can propagate disease in a secondary recipient (most frequently an immune-deficient mouse), produce cellular progeny in more than one cell lineage, and can be prospectively defined within, and isolated from, the original tumor population [17]. In contrast to very low frequencies of LICs typically defined in AML and CML, LICs from ALL (including Ph+ ALL), are more abundant within the primary leukemia [18–21] and resemble more differentiated lymphoid progeny (i.e. not derived from true hematopoietic stem cells) that have subsequently acquired self-renewal capacity [18,20,21]. As contemporary anti-leukemic chemotherapy regimens successfully de-bulk the abundant differentiated leukemic cell progeny within tumors but often fail to eradicate the residual drug-refractory LICs that later contribute to clinical relapse, the most successful future therapeutic strategies should target and eradicate LICs.

However, there remain significant challenges on the path to clinical deployment of these cytokine–toxin fusions in high-risk ALL. DT₃₈₈IL3 or enhanced derivatives will require careful single-agent and combination therapy efficacy testing in either syngeneic and/or xeno-transplanted murine ALL models, as has been successfully undertaken for targeting of IL3-expressing AML cells with either IL3-DT toxins [8] or anti-IL3 receptor antibodies [16]. Toxicities associated with single-agent DT₃₈₈IL3 treatment in human patients with AML or myelodysplasia are manageable and include constitutional (fever, chills); cardiovascular (hypotension and vascular leak syndrome); hepatic (elevated transaminases, hypoalbuminemia); and metabolic (hypocalcemia) abnormalities [12], suggesting that IL3-DT toxins might be cautiously advanced into clinical testing. Finally, the development of neutralizing anti-DT₃₈₈IL3 antibodies, especially after multiple cycles of therapy might impair their effectiveness, although limited published experience does not support this concern [7].

DT₃₈₈IL3 toxin represents an innovative strategy to target and eradicate IL-3 receptor-expressing lymphoblasts. The study of Kim et al. [4] provides a strong rationale to move DT₃₈₈IL3 into pre-clinical testing against lymphoblastic leukemias, particularly in model systems that are well situated to test multi-agent combination therapies including conventional agents and TKIs. Despite recent optimism that multi-agent combination chemotherapy that includes imatinib can provide at least medium-term disease control in a significant fraction of Ph+ ALL patients [3], innovative and clinically meaningful therapeutic strategies are still desperately needed.

Conflict of interest

Dr Williams declares no relevant conflict of interest.

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