methodically structured joint reflection among health care professionals when a case that is perceived as morally or ethically troublesome.

In time, hopefully more products and alternative manufacturing approaches like point of care production and decreased costs may improve access to CAR T-cell therapies worldwide. Also, alternative effective therapies such as bispecific antibodies may in part replace CAR T-cell therapy as they are available "off the shelf." In addition, evidence-based algorithms to predict individual efficacy, outcome, and toxicity risk would help in prioritizing patients. Unfortunately, such an algorithm is not yet available, and algorithms do have limitations. Currently patient prioritization depends on multidisciplinary decision-making, and the results of the study by Bell et al help by offering a well-described ethical framework, highlighting transparency and equity, which is an important first step toward uniformity in prioritizing patients for CAR T-cell therapy.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Brammer et al, page 1271

Blocked addiction to IL-15 for treating T-LGLL

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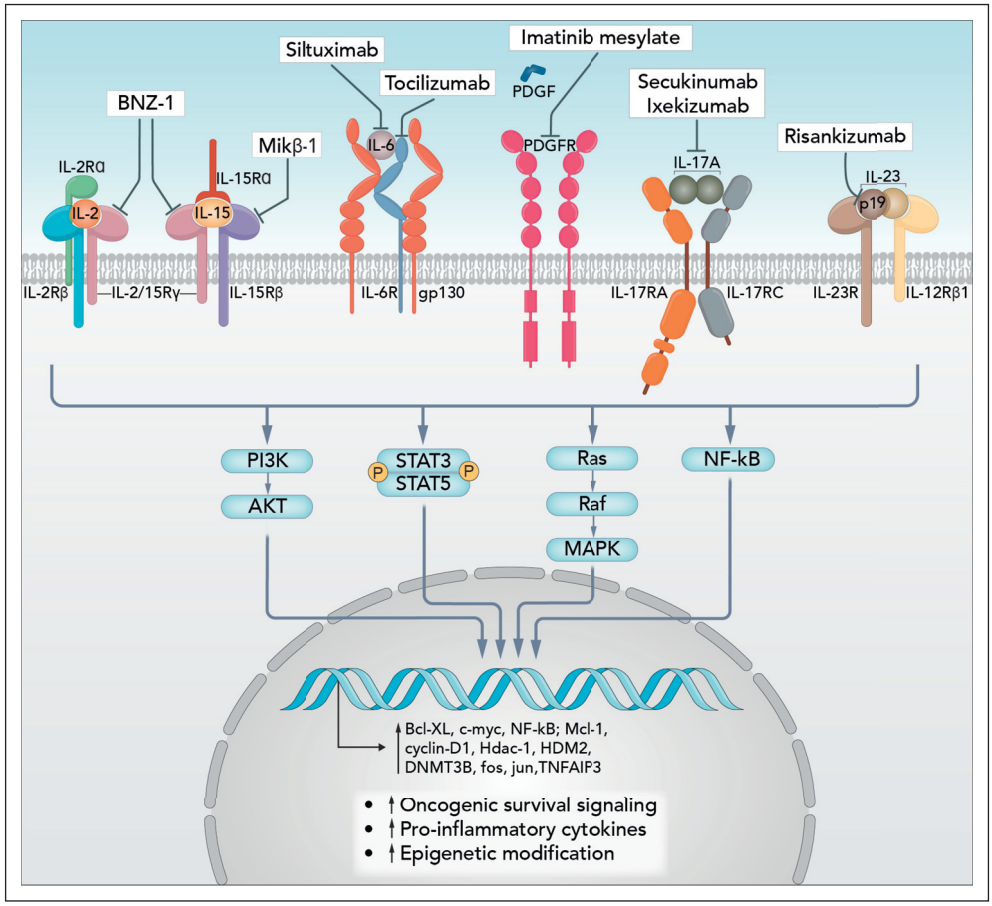
In this issue of *Blood*, Brammer et al present a phase 1/2 trial that demonstrates in patients with T-large granular lymphocyte leukemia (T-LGLL) that blocking interleukin-15 (IL-15) binding to CD132 using the pegylated peptide BNZ-1 results in the resolution of cytopenias in 20% of patients.[1] This is the first trial that has the potential to advance our treatment options beyond the current approach of using either methotrexate, cyclophosphamide, or cyclosporine.

Large granular lymphocyte (LGL) leukemia is a chronic leukemia due to the clonal expansion of LGLs, which can be either cytotoxic T lymphocytes (CTLs) or natural killer (NK) cells.[2] The World Health Organization classification differentiates T- from NK-cell LGL leukemia. Normal T-LGLs become activated through antigen recognition and undergo significant expansion with subsequent death by apoptosis upon antigen clearance. In LGLL, the LGLs persist and infiltrate the blood, marrow, and spleen.[3,4] T-LGLL is a relatively unique condition in which, unlike most lymphoproliferative diseases, the indication for treatment is not disease bulk but rather the resultant cytopenias—particularly neutropenia and anemia and less commonly pure red cell aplasia, aplastic anemia, and thrombocytopenia.[2]

T-LGLL is thought to be triggered by chronic antigen stimulation that drives cytotoxic T cells to develop a terminal effector memory phenotype that transforms to a clonal proliferation.[5] During disease development, T-LGLL cells may acquire the ability to sustain proliferative signaling by either producing growth factors and their cognate receptors themselves, resulting in chronic autocrine proliferative stimulations, or by responding to soluble growth factors present in a proinflammatory microenvironment. The most important cytokines driving this process are IL-15, platelet-derived growth factor (PDGF), IL-2, and IL-6. The interactions of these cytokines and the downstream oncogenic signaling drivers active in T-LGLL, that is, Jak/STAT, Ras-Raf-1-MEK1ERK/MAPK, Pi3k/Akt, nuclear-factor κB, Fas, and Fas ligand (FasL) signaling, and the sphingolipids sphingosine-1-phosphate and ceramide pathways, lead to increased transcription of oncogenic driver genes such as *c-MYC*, *cyclin D1*, and *BCL-xL*, culminating in increased malignant cell proliferation and survival (see figure).[6]

IL-15 signals through a heterotrimeric receptor that include (1) the IL-15-specific receptor subunit, IL-15Rα; (2) IL-2/IL-15Rβ (CD122), ie, shared with IL-2; and (3) the common gamma-chain (CD132) that is shared with the IL-2, IL-4, IL-7, IL-9, and IL-21 receptors. This in turn activates downstream signals including Jak/STAT and MAPK, which suppress proapoptotic factors in the Bcl2 family.[7] In vitro and mouse models targeting IL-15 demonstrated that blocking IL-15 signaling was able to suppress LGL leukemia.[7] Constitutive activation of STAT3 has been demonstrated as a unifying feature in T-LGLL, promoting LGL survival through regulation of anti-apoptotic proteins.[8] Moreover, about 30% to 40% of patients with NK and T-LGL have somatic activating mutations in the *STAT3* gene, and a few percent have *STAT5b* mutations.[9]

This trial in patients with T-LGLL with a terminal effector memory phenotype and significant cytopenias is the first to demonstrate the potential of targeting IL-15, using BNZ-1 to target the IL-15 receptor common gamma chain (CD132). The authors found that 87% of evaluable patients showed apoptotic response to BNZ-1 treatment, and



Cytokine pathways involved in survival of large granular lymphocytes and examples of mechanisms of inhibition. Professional illustration by Somersault18:24.

20% of patients had objective clinical responses in their cytopenias including transfusion independence and resolution of neutropenia. These responses occurred rapidly and were durable, with one lasting beyond 13 months. A prior study of a humanized antibody to IL-15, Hu-Mikβ1, demonstrated no therapeutic efficacy.[10] Unlike BNZ-1, the Hu-Mikβ1 antibody was directed at the shared IL2/IL-15Rβ subunit (CD122) and blocked the trans presentation of IL-15 to CD122 on T cells but did not block IL-15 action in cells that expressed the heterotrimeric IL-15 receptor in cis.

The apoptosis studies in the Brammer et al trial clearly demonstrate that T-LGLL cells are dependent on IL-15 in vivo. Moreover, in those responding patients, apoptosis was maintained throughout the treatment period, suggesting that these cells remain dependent on IL-15, and thus are sensitive to ongoing BNZ-1 therapy. The lack of any difference of response due to *STAT3* mutation status is likely explained by *STAT3* mutations not rendering the cells IL-15 cytokine independent, but rather more sensitive to IL-15. The studies also demonstrate that in some patients, the LGLs lose their dependence on IL-15, likely due to other signaling pathways predominating, either driven by other cytokines or being cytokine independent.

The evaluation of response was not based on bulk reduction of the T-LGLL but on resolution of cytopenias, and response criteria were based on the large Eastern Cooperative Oncology Group E5998 trial. However, the exact mechanism by which the cytopenias resolved with BNZ-1 therapy has not been determined. The authors did not explore the Fas/FasL signaling pathway, which is known to be important in T-cell apoptosis and the cytopenias associated with T-LGLL. This clearly requires further investigation.

BNZ-1 is a rationally developed drug that has demonstrated efficacy despite the failure of a previous attempt at targeting the receptor for IL-15. Like in Castleman disease, in which we know an addiction to the IL-6 pathway is critical to prosurvival of the lymphoid cells, it has been proven here for the first time that a proportion of patients with T-LGLL are dependent on IL-15. However, given the modest response rate with BNZ-1 alone, it is clearly not the whole answer to managing this disease. As outlined in the figure, there are other rational targets for treating T-LGLL, including inhibitors of the other cytokines such as PDGF, IL-6, IL-17, and IL-23 and critical pathways such as Jak/STAT. Combinations of BNZ-1 with drugs that target these clearly warrant investigation.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Xiao et al, page 1312

BMP5: a novel tile of the hepcidin regulatory pathway

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In this issue of *Blood*, Xiao et al[1] describe, for the first time, a role for bone morphogenetic protein 5 (BMP5) in the control of hepcidin, the master regulator of systemic iron homeostasis.

Hepcidin is a peptide hormone produced by the liver, which limits iron absorption from the diet and iron release from stores. Hepcidin acts by occluding and degrading the sole iron exporter, ferroportin. Hepcidin is transcriptionally regulated by different pathways, among which a major role is played by the BMP suppressor of mothers against decapentaplegic (SMAD) system. On BMP's binding, the BMP type II receptors (BMP receptor 2 and activin A receptor type 2A (ACVR2A)) phosphorylate type I receptors (activin receptor-like kinase 2 (ALK2) and activin receptor-like kinase 3 (ALK3)), which, in turn, phosphorylate SMAD1/5/8. These proteins bind the cargo SMAD4; then, the complex translocates into the nucleus to activate the transcription of genes carrying a BMP-responsive element, including hepcidin.[2]

Two BMP ligands mainly produced by liver sinusoidal endothelial cells (LSECs), BMP2[3,4] and BMP6,[5,6] are relevant in hepcidin modulation. BMP2 maintains basal hepcidin levels binding ALK3, whereas BMP6, upregulated by liver iron, activates the pathway in conditions of tissue iron accumulation, preferentially via ALK2.[2] Despite the crucial and nonredundant roles of these 2 ligands, iron is still able to activate the BMP-SMAD-hepcidin pathway in double LSEC *Bmp2* knockout (KO) and global *Bmp6* KO mice, suggesting that at least 1 additional ligand is involved in the upregulation of hepcidin. In the present study, Xiao et al demonstrate that BMP5 is likely the missing piece of the puzzle.

The role of BMP5 in the physiological regulation of hepcidin is marginal compared with that of BMP2 and BMP6. Indeed, mice lacking *Bmp5* in the whole organism do not show dysregulation of the iron regulatory system, with the sole exception of a modest decrease in hepcidin levels when 10 days old. This is potentially reminiscent of a defect during fetal life, which is resolved in adulthood. However, when these animals are challenged with an iron-poor or a high-iron diet, they accumulate more iron in the liver and maintain inappropriately low hepcidin levels. This finding proves that BMP5 contributes to the transcriptional activation of hepcidin in response to both low and high iron, when *Bmp6* is virtually absent or maximally induced. In agreement, *Bmp5* inactivation in mice lacking *Bmp6* (both globally or specifically in LSECs) dramatically reduces hepcidin levels, worsening both hepatic and extrahepatic iron overload, an effect exacerbated by feeding animals a high-iron diet (see figure). In *Bmp6* total KO, the loss of *Bmp5* causes an increased mortality, irrespective of the degree of iron overload, which is comparable in both *Bmp6* global and LSEC-specific KO, but likely related to a redundant nonendothelial developmental role of BMP6 and BMP5, which remains to be elucidated.

At difference with BMP2 and BMP6, which are mainly produced by LSECs in response to iron levels, *Bmp5* is not preferentially expressed in any liver cells and is not transcriptionally activated by iron. However, its activity appears strongly dependent on iron availability, raising the possibility that the metal controls BMP5 at the posttranscriptional level. However, the lack of specific antibodies currently precludes the possibility of addressing this point. Also, how BMP5 works, which receptors it uses, whether it can form heterodimers with BMP2, BMP6, and/or other BMPs, its potential involvement in the fetal iron homeostasis, and why BMP6 levels are limiting for the function of BMP5 are still unsolved issues, which likely will be the focus of further studies from the same and other groups in the field.

BMPs are crucial not only for the iron-mediated regulation of hepcidin, but also for hepcidin control by the expanded erythropoiesis. In response to erythropoietin (EPO) stimulation, developing erythroblasts produce the erythroid regulator erythroferrone (ERFE), which inhibits hepcidin transcription to increase iron supply needed for hemoglobin production.[7] ERFE functions by binding and sequestering BMPs, mainly BMP6, but also BMP5 and BMP7.[8] However, EPO injection is still able to suppress hepcidin in *Bmp6* global KO,[9] proving that other BMPs likely contribute to the ERFE-mediated hepcidin downregulation. The current study demonstrates that BMP5 is involved in this