of GVHD. They report on 28 adult patients who participated in a multicenter phase 1/2 clinical trial, TK007. In this trial, purified mature donor-derived T cells that were retrovirally transduced with the Herpes Simplex Thymidine Kinase suicide gene (TKpos cells) were serially infused into adult patients with various hematologic malignancies after myeloablative conditioning and transplantation of highly purified CD34+ stem cells from haploidentical donors. In case of occurrence of GVHD, the suicide mechanism can be activated by ganciclovir to abrogate GVHD. Twenty-two of the 28 patients who received the purified TKpos cells showed a rapid recovery of T cells that was associated with a concomitant improvement of the clinical outcome and reduction of transplant-related mortality. Patients not receiving TKpos cells failed to attain T-cell immune reconstitution and had a dismal outcome mainly due to infectious complications. Therefore, the infusion of TKpos T cells seemed to induce a rapid and robust T-cell reconstitution. Interestingly, further follow-up of these patients revealed that the regenerating CD3+ T cells failed to express the TK suicide gene (TKneg cells), while donor-derived TKneg cells were not detected in patients who did not receive genetically modified T cells nor in those who failed to engraft TKpos cells. The TKneg cells were enriched in the CD4+ T-cell subset and an increase of the T-cell subset with a naive phenotype (CD62L+CD45RA+) was seen during the observation period even in elderly patients. In addition, measurement of the sjTREC counts in patients treated with the TKpos cells demonstrated an increase of sjTREC counts parallel to the increase in the naive T-cell subset. Strikingly, an increase of the serum levels of IL-7 was observed early after TKpos T-cell add-backs followed by a concomitant rise in peripheral T-cell counts. Further analysis of the reconstituting naive TKneg cells revealed that most of the TKneg naive CD4 cells co-expressed CD31, indicating a continuous output of RTEs, compatible with the computed tomography-based observation of increased thymic tissue even in elderly patients (see figure). Additional experiments demonstrated that the antiviral activity of the newly generated TKneg cells was fully maintained even after elimination of the TKpos cells through the activation of the suicide mechanism. The biologic mechanism for these unexpected findings is unclear. The transient increase of IL-7 might contribute, but is most likely not sufficient to induce long-lasting thymopoiesis. The role of the retroviral transduction of the T cells with the suicide gene remains to be shown and it might well be that the ex vivo stimulation of donor T cells with IL-2 and anti-CD3 antibody used for preparation of the cells for transduction induced a shift toward memory T cells, which have been shown to be less responsive to alloantigen stimulation compared with naive T cells. Because the incidence of GVHD was surprisingly low after add-back of high numbers of transduced T cells, further clinical dose-escalating studies with ex vivo activated but not transduced T cells might be envisioned to investigate the role of TK transduction on thymic function.

The impressive long-lasting increase of naive T cells and the continuous generation of RTEs warrant further clinical trials. The observations by Vago et al might offer new approaches to accelerate immune recovery in patients not only after haploidentical transplantation of CD34+ stem cells but also in other transplant settings, especially in elderly patients.

Conflict-of-interest disclosure: The author declares no competing financial interest.

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Comment on Tai et al, page 1877

BTK inhibition in myeloma: targeting the seed and the soil

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In this issue of Blood, Tai and colleagues identify Bruton tyrosine kinase (BTK) inhibition as a new and effective strategy for the treatment of myeloma and the associated bone disease.1

Despite many recent advances in our understanding of the pathogenesis of multiple myeloma and the discovery of powerful agents such as thalidomide and bortezomib, myeloma still remains a fatal malignancy. The reciprocal relationship that develops between myeloma cells (the “seed”) and cells of the host microenvironment (the “soil”) results in a vicious cycle that exacerbates both tumor growth and the development of the osteolytic bone disease (see figure).2,3 Thus, an optimal drug would not only have direct antilymphoma effects but would also act on the host micro-environment to disrupt this relationship resulting in indirect antitumor effects and/or prevention of the osteolytic bone disease.

BTK is a nonreceptor tyrosine kinase that is expressed in many hematopoietic lineages
Effect of BTK inhibition in the bone microenvironment in multiple myeloma. Myeloma cells promote the development of the associated bone disease, and the bone disease promotes tumor growth and survival, resulting in a vicious cycle of increased tumor burden and increased osteolytic bone disease. BTK inhibition has multiple effects to (1) directly inhibit tumor growth, (2) directly inhibit osteoclastic bone resorption, (3) inhibit the release of osteoclast-derived tumor growth factors, and (4) prevent adhesion to bone marrow stromal cells (BMSCs) and release of BMSC-derived growth factors. The culmination of these effects is to reduce tumor burden and osteolytic bone disease. Professional illustration by Alice Y. Chen.

and plays a critical role in B-cell maturation. Targeting BTK in chronic lymphocytic leukemia (CLL) has been shown to have potent antitumor effects, leading to the clinical investigation of an oral, irreversible, selective BTK inhibitor, PCI-32765 (Ibrutinib). Tai and colleagues demonstrate strong expression of BTK in malignant plasma cells from patients with myeloma. This observation combined with the recently discovered role for BTK in the promotion of osteoclastic bone resorption, led them to hypothesize that BTK inhibition may disrupt multiple components of the vicious cycle and, as such, represent an ideal target in multiple myeloma. To investigate this, they used the BTK inhibitor PCI-32765 in a series of elegant in vitro studies designed to reflect the cellular interactions within the bone marrow microenvironment and finally in an in vivo model of myeloma.

The knockdown of BTK expression or treatment of myeloma cells directly with PCI-32765 resulted in a modest reduction in cell viability and induction of apoptosis, an expected result consistent with other hematopoietic malignancies. Further enthusiasm for the direct potential of BTK inhibition was generated by the ability of PCI-32765 to suppress the clonogenicity of myeloma stem-like cells, a subpopulation of cells postulated to contribute to chemo-resistance and disease relapse. In addition to these promising effects of PCI-32765 on the seed, Tai and colleagues also examined the effect of BTK inhibition on the soil. PCI-32765 reduced osteoclast formation and bone resorption, supporting the direct role for BTK in osteoclast bone resorption previously identified in genetically modified mice. The release of tumor growth factors, either from osteoclasts, bone marrow stromal cells (BMSCs), or resorbed bone, is an important component of the vicious cycle. PCI-32765 was found to reduce cytokine and chemokine secretion from both osteoclasts and BMSCs, with inhibition of a number of key myeloma growth factors, including a proliferation-inducing ligand, activin A, macrophage inflammatory protein-1α, transforming growth factor−β, and stromal cell–derived factor. Working concomitantly with the release of tumor growth factors to perpetuate the vicious cycle is the release of so-called “osteoclast activating factors” from myeloma cells, and it would have been of interest to determine whether BTK inhibition had similar effects to inhibit secretion of these factors. Further evidence for BTK inhibition to target multiple cellular interactions within the tumor:bone microenvironment was provided by the enhanced cytotoxicity of PCI-32765 when myeloma cells were cultured in the presence of BMSCs and the reduction in adhesion and migration of myeloma cells to BMSCs in response to BTK inhibition.

These in vitro studies provide compelling evidence to support the potential for targeting both seed and soil by BTK inhibition. However, it is impossible to fully recreate the complexities of the bone marrow microenvironment in an in vitro setting, necessitating the use of animal models that accurately reflect human disease. The response to PCI-32765 was evaluated in a severe combined immunodeficient (SCID)−hu model, in which myeloma cell growth is restricted to human bone implanted into immunodeficient mice and associated with osteolysis of the human bone fragment. BTK inhibition was found to reduce both tumor burden and osteolytic bone disease, associated with a reduction in osteoclast number and activity. Although strongly supportive of a positive effect of BTK inhibition in the treatment of myeloma disease, the study design, treatment from time of tumor detection, does not reflect the clinical situation where the majority of patients present with osteolytic bone disease on diagnosis with myeloma. Thus, it remains to be determined whether BTK inhibition is still effective in a preventative setting when osteolytic lesions are already present, particularly because this approach appears to have minimal effect on osteoblastic bone formation.

The conclusions by Tai and colleagues both identify a novel role for BTK in myeloma pathogenesis and provide strong support for the rationale for targeting BTK in this hematologic malignancy. These studies are made all the more exciting by the use of the BTK inhibitor PCI-32765, which is currently undergoing clinical evaluation in CLL, with initial reports demonstrating good safety profiles and response rates. Furthermore, the ability of PCI-32765 to target both tumor growth and osteolysis render it an extremely attractive approach for the treatment of myeloma and the associated bone disease.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Comment on Ranganathan et al, page 1765

**A drug that stops traffic at the nuclear border**

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In this issue of Blood, Ranganathan et al seek a holy grail: evidence that a drug with a novel mechanism of action may be effective for acute myeloid leukemia patients.\(^1\)

Unfortunately, the overall remission rate for adult patients with acute myeloid leukemia (AML) is only approximately 40%, and has remained at that level for several decades despite great effort into developing new drugs. So, the prospect of a new approach is welcome news.

In order for proteins to function normally, they must be found within their proper subcellular compartment. In cancers, one way to disrupt protein function is to mis-localize it within the cell. For example, in AML the NPM mutation, found in almost 30% of all adult AML cases,\(^2\) results in a protein that fails to shuttle normally between the nucleus and cytoplasm, instead becoming localized inappropriately to the cytoplasm. Although more than 50 different NPM mutations have been identified in AML samples, they all result in unfolding of the C-terminus and the creation of a novel nuclear export motif recognized by CRM1 (XPO1, exportin), a protein found within the nuclear membrane that controls the export of various proteins (eg, NPM, p53, p21, topoisomerase II, and NF-kB/1-kB) into the cytoplasm (see top panel of figure). These consequences are so reliable that pathologists can use the cytoplasmic localization of NPM as a surrogate for molecular mutation testing.

The idea for a new drug, then, is simple: If proteins that are supposed to work in the nucleus are disrupted by merely sequestering them in the cytoplasm, an effective strategy might be to concentrate the protein in the nucleus, where it may function properly. To block the export of proteins out of the nucleus, researchers surmised the key residues required for the transport function of CRM1 by studying the known 3-dimensional structure of CRM1 with its cargo.\(^3,4\) With this information in hand, Karyopharm Therapeutics developed selective inhibitors of nuclear export (KPT-SINE), orally bioavailable small molecules that bind to the critical Cys528 site within CRM1 and irreversibly block the eflux of proteins into the cytoplasm, effectively concentrating them in the nucleus where they can potentially mediate apoptosis in response to chemotherapy (see bottom panel of figure).

Ranganathan and colleagues present the first in vitro data showing that KPT-SINE has efficacy against AML.\(^1\) KPT-SINE suppressed the proliferation of a variety of AML cell lines and samples of primary AML cells of several genotypes, inducing cell-cycle arrest in G1 and/or apoptosis as well as differentiation of several AML cell lines. As expected, KPT-SINE treatment resulted in nuclear accumulation of p53 and NPM, both within cell lines and primary blasts. Interestingly, the group observed a decrease in both FLT3 and KIT protein levels after drug treatment, with no effect on their respective mRNA levels, suggesting a posttranscriptional effect. To test the drug’s effect in vivo, Ranganathan et al established mouse xenografts using the MV-4-11 cell line, which carries the FLT3 internal tandem duplication (ITD) mutation. One week after the mice were inoculated with the leukemic cells, half of the cohort received a KPT-SINE with excellent bioavailability. Treated mice survived longer and had a lower leukemic cell burden than
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