not able to receive all 6 cycles because of prolonged cytopenias. In the study by Kasamon et al, hospitalization occurred 27 times among 16 patients during or within 2 months after chemotherapy for a variety of causes, including febrile neutropenia (n = 7), nonneutropenic infections (n = 9), thrombosis (n = 4), pulmonary toxicity (n = 3), and other causes (n = 4), an unusually high rate of complications compared with large studies of advanced cHL or smaller retrospective analyses.

Interestingly, only about 20% to 30% of cHL cells express the B-cell antigen CD20,[3] the target for rituximab in most B-cell non-Hodgkin lymphomas (NHLs). In the study by Younes et al CD20 was expressed in 20%, while in the study by Kasamon et al only in 8%. So much for targeting cancer. Yet that was not the point of these studies, as the target was not the HRS cell (not the malignant cell as we have come to expect in NHL and in other cancers), but the clonotypic stem cell (Kasamon et al) or the B cell harbored in the microenvironment (Younes et al). Therein lies the importance of these trials. The concept of "off-targeting" the HRS cell is appealing if one looks at the biology of cHL. The data from Jones et al documenting the presence of a $CD20^+$ clonotypic B cells in cHL suggests a potential target for rituximab, but the low frequency of these cells in the circulation may limit utility.[8] Frequency of clonotypic B cells was measured in the Kasamon study reported here. Of the 24 patients with baseline levels, 88% had detectable clonal $CD27^+ALDH^{high}$ B cells before rituximab or rituximab-ABVD, representing 0.3% of total circulating $CD19^+$ cells. After treatment they became undetectable in 19 patients. It would be informative to know if levels were measured after rituximab alone. Three of these 19 had re-emergence of the clone but are still in remission. The microenvironment in cHL may provide another potential "off target" for CD20-directed therapy.[11] In solid tumors, data are accumulating that B-cell depletion may help to retard tumor growth. Kim et al have shown that in murine tumor models, depletion of $CD20^+$ B cells by a mouse anti-CD20 antibody slowed the growth of new $CD20^-$ solid tumors and retarded the growth of established tumors but did not induce tumor regression.[12] However, when an active immunotherapy approach using an adenovirus vaccine bearing the human papilloma virus E7 was combined with B-cell depletion against a murine lung cancer expressing the E7 virus, there was enhanced tumor regression and an immune response as measured by increased $tetramer^+$ $CD8^+$ T cells. Further, work from de Visser and colleagues has demonstrated that de novo carcinogenesis related to chronic inflammation depends on B-lymphocytes, and data are accumulating that suggest that B cells may inhibit Th-1 mediated anti tumor immune responses.[13]

Approximately 20% to 40% of cHLs harbor EBV in the HRS cells, and EBV has been shown to predict for a negative outcome in older patients. EBV can be detected in whole blood, plasma, and mononuclear cells by real-time PCR for viral DNA (reviewed in Küppers[3]). Correlates for EBV in HRS cells found high sensitivity (90%) and low specificity (65%), but interestingly, plasma EBV-DNA at diagnosis was an indicator of disease activity and correlated with expression of $CD68^+$ macrophages,[14] known to be an independent negative prognostic factor in cHL.[15] In the Kasamon study, EBV copy number was measured and fell dramatically during cycle 1 in patients with EBV^+ tumors.[10]

As we begin to unravel the underlying biology of cHL and other malignancies, it seems likely that our approach will be different and subtler. That subtlety underlies the importance of these two papers. More correlative studies looking at "off-target" effects of new agents and ultimately randomized clinical trials designed to test the biology will be needed, but these two articles provide an important statement if not proof of principle.

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Comment on Pegram et al, page 4133

The power and pitfalls of IL-12

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In this issue of *Blood*, Pegram and colleagues describe the ability to improve adoptive T-cell therapies by engineering T cells to secrete the potent proinflammatory cytokine IL-12.[1] These exciting findings significantly extend previous observations made in a murine melanoma model targeting naturally occurring tumor antigens.[2,3]

Despite its discovery more than 20 years ago and the flurry of research related to its biologic function, IL-12 remains a controversial cytokine as a therapeutic anticancer agent.[4] The benefits of administering IL-12 systemically were demonstrated in multiple preclinical studies through the early 1990s, but these promising findings never translated into successful human clinical trials.[5] One of the major issues that limited the clinical development of IL-12 were the reported dose-limiting toxicities, which included fevers, elevated hepatic enzymes, hemodynamic instability, and in some cases death.[4]

Notwithstanding these setbacks, the importance of IL-12 for inducing an immune response against cancer is undeniable and the quest for using this potent cytokine for the immunotherapy of cancer continues.[5,6] The findings by Pegram et al show a translatable method for harnessing the beneficial effects of IL-12 that may also minimize the hazardous outcomes associated with its use (see figure).

CD19 is an attractive tumor antigen target because it is robustly expressed by some leukemias and lymphomas. It is expressed by normal B lymphocytes, but not by other vital tissues. Although depleting CD19 results in the ablation of B cells, patients can be treated with infusions of immunoglobulins. CD19 has been recently targeted by a number of groups, including Brentjens.[7,8] This approach is encouraging and it is conceivable that gene-engineered T cells targeting CD19 might eventually replace allogeneic bone marrow transplantation as a curative regimen for a subset of patients with B-cell malignancies. The use of a single-chain functional IL-12 molecule represents a welcome addition to this therapy, because it may eliminate the need for lymphodepletion and high-dose IL-2 used in current regimens.

In their study, Pegram and colleagues show the critical need for autocrine production of IL-12 and IFN-γ to enhance the effects of adoptively transferred T cells. They also suggest that $CD4^+$ T cells expressing IL-12 enhance the function of co-transferred $CD8^+$ T cells and may be important for overcoming negative regulatory factors induced by tumors.[1]

Additional mechanisms ascribed to IL-12 include the ability to functionally reprogram myeloid-derived cells. Growing tumor masses can create an immunosuppressive microenvironment orchestrated by cellular infiltrates such as myeloid-derived suppressor cells, alternatively activated macrophages, and functionally impaired dendritic cells. Surprisingly, the majority of cells expressing the functional IL-12 receptor-β2 subunit within the tumor microenvironment are of myeloid origin.[2] Sensitization of these cells by IL-12 likely triggers an acute inflammatory environment that enables improvements in antigen presentation and T-cell co-stimulation within tumors.[2]

Interestingly, Pegram et al noted that they did not observe toxicities in this study, although targeting human CD19 in this complex mouse might not fully capture all the toxicities. Others have observed toxicities from IL-12 production by T cells[3] and so the issue might be seen in future clinical trials. It is difficult to predict the replicative potential of transferred T cells because every daughter cell of the gene-engineered T cell will also be producing supra-physiologic amounts of IL-12. Current safety measures that are being explored include the use of an inducible NFAT promoter, incorporation of suicide genes, or the use of molecular on/off promoter switches controlled by the systemic administration of a third-party drug.[9,10] Carefully conducted clinical trials will be needed to assess whether the benefits of gene engineering T cells with IL-12 outweigh the risks.

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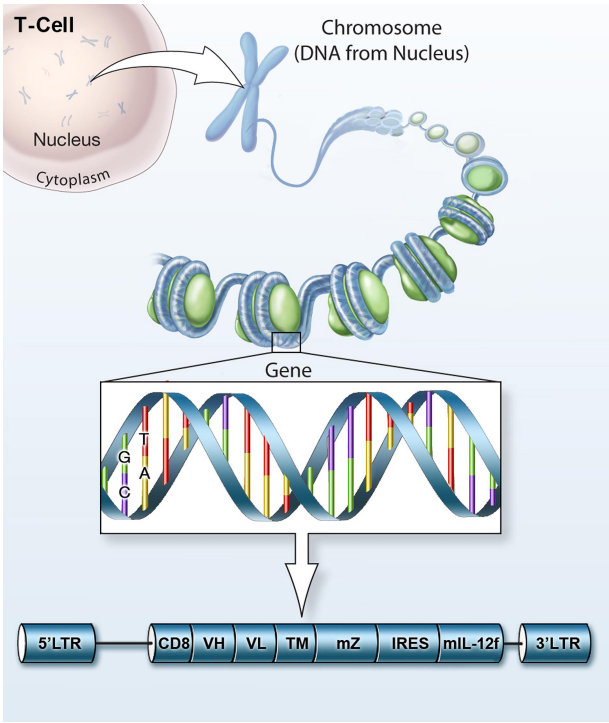

Gene-engineered T cells co-expressing CD19 CAR and IL-12 for adoptive cell therapy against cancer.

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