have noted before, it is known that different tumor mutations can influence susceptibility to OV.[7] Although it is tempting to hope that a single virus could serve as an off-the-shelf treatment for all cases of GBM, realistically, tumor mutations influencing OV-mediated lysis may need to be identified and individual tumors may need to be matched to particular oncolytic viruses more efficacious for those genomic phenotypes. Furthermore, even with optimized delivery of virus and widespread infection, not all tumor cells may be successfully infected and subject to direct viral cytotoxicity. One of the key tenets of the OV paradigm is that the widespread death of tumor cells in the context of the inflammatory response may induce immune specificity to newly exposed tumor antigen, resulting in the priming of virally induced antitumor immunity.[4] Although discovering the means to augment antitumor immunity induced by OV is an important direction for this modality, it is critical to first maximize the direct viral-mediated lysis of tumor cells not only to eradicate malignancy but also to optimize the inflammatory conditions and exposure of tumor antigen that will engender antitumor immunity. The work of Kim *et al*. demonstrates a substantial improvement in the OV of glioma, and we look forward to the translation of this work to the clinic.

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CAR T Cells for Acute Myeloid Leukemia: The LeY of the Land

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Recent studies of CD19-directed chimeric antigen receptor (CAR)-expressing T cells have shown dramatic results in the treatment of acute lymphoblastic leukemia as well as activity in B-cell lymphoma.[1–3] These highly publicized studies have led to the hope that CAR T cells can be used to treat other hematological malignancies and solid tumors as well. The article by Ritchie *et al.* in this issue[4] shows how the approach can be adapted for the treatment of patients with acute myeloid leukemia (AML) and illustrates that significant obstacles remain before this biological therapy will become a mainstay of treatment.

Although CARs directed to the CD19 antigen on lymphoid malignancies are proving to be highly active, there has been concern that it may be difficult to extend this success to many other tumors. The CD19 antigen is restricted to the B-cell lineage in general; it is not limited to the malignant clone. Eliminating a CD19$^+$ malignancy therefore also leads to the eradication of normal B cells. There are few other examples of normal cell lineages that can be safely eliminated along with malignant cells that share the targeted antigen. In particular, targeting an antigen on AML cells that is also present on the normal myeloid lineage would lead to profound neutropenia, which would have devastating effects on a patient's health even if his or her AML had been eradicated. Investigators have therefore long sought antigens that are either highly overexpressed on myeloid malignancies compared with normal cells or unique to the malignant myeloid clone. Several such antigens have been identified, but the great majority are internal proteins that are processed and then presented as peptides by the cells' human leukocyte antigens.[5] Such peptides can usually be recognized only by the native T-cell receptor, not by an antibody-derived CAR, although monoclonal antibodies that recognize a major histocompatibility complex (MHC)-peptide complex have recently been synthesized.[6,7] Ritchie *et al.*[4] therefore took advantage of one of the defining characteristics of antibody derived CARs, which is that they are MHC-unrestricted and can recognize nonprotein antigens. They developed a CAR that targeted LeY, a difucosylated carbohydrate antigen that is overexpressed by malignant myeloid cells. Following treatment with cytotoxic drugs, they infused T cells expressing this CAR into five patients with relapsed AML, four of whom were evaluable. Modest CAR T-cell expansion and persistence was seen, and two of the four patients had a reduction in their residual disease, although all ultimately relapsed.

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Although the clinical responses in this study were limited, the results have important implications. The data provide a clinical proof of concept for using CARs to target nonprotein tumor–associated antigens in hematological malignancy, as in solid tumors.[8] The data also demonstrate good tolerance of modest levels of CAR T-cell expansion and persistence, although one patient was reported to have neutropenia as an adverse effect. The LeY antigen was not measurably downregulated following CAR T-cell infusion, suggesting that it may indeed serve as a suitable target for long-term immune control of disease. Most important, perhaps, the article demonstrates that the CAR T cells can traffic to the bone marrow and also to disease sites in skin, raising the possibility that the approach may ultimately eliminate both central (e.g., marrow) and peripheral (e.g., skin) reservoirs of disease.

Almost all first-in-human proof-of-concept studies raise as many issues as they address, and the work described in the current article is no exception. It is clear that the degree of *in vivo* expansion of the CAR T cells and the level at which they persist *in vivo* are likely to be below those required for effective and sustained control of AML. There are many modifications to the CARs themselves that may improve expansion, persistence, and antitumor function *in vivo*, including the use of alternative costimulatory endodomains and alterations in extracellular spacer and transmembrane sequences.[9] The procedures for growing the CAR-transduced T cells can also have profound effects on their *in vivo* performance, not least by selecting subsets that have little long-term engraftment potential or that are "exhausted" and subject to antigen-induced cell death.[9] In addition, a more sensitive means of tracking transgene expression *in vivo* will need to be developed for this CAR. The authors were able to follow the CAR T cells only by detecting a transgene-derived PCR signal, which does not by itself show that the transduced T cells express the CAR and can still recognize their intended target. Finally, the CAR T cells may need to be further engineered to resist tumor-immune evasion strategies[10] or to be administered with antibodies that serve as checkpoint inhibitors and prevent CAR T-cell inactivation.[11] All these components will need to be optimized before we will be able to accurately evaluate the benefits of the approach.

Optimization of this therapy may, of course, reveal new problems. Rapid and substantial *in vivo* expansion may lead to a systemic inflammatory response syndrome or cytokine storms encountered with other more effective CART-cell therapies,[2] and which were notably absent in the current series. Once the CAR T cells are present at high levels for long periods, we may learn that selective overexpression of LeY is not quite selective enough and that critical normal tissues, such as hemopoietic stem cells, are also damaged. The availability of effective safety or suicide systems may help address this last concern.[12]

Despite the above limitations, this important clinical proof-of-concept study has shown that CAR T cells can target a nonprotein target antigen in patients with myeloid malignancy; further optimization and extension to other LeY$^+$ tumors may well lead to more striking clinical benefits

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