However, if azithromycin is going to be used in patients who are known to have frequent exacerbations of COPD, then the local antibiotic resistance patterns should be closely monitored. It also makes sense to ask whether, in such patients, subsequent exacerbations should be treated empirically with a different class of antibiotics. On balance, however, the long-term use of azithromycin to prevent acute exacerbations of COPD would not seem to be at odds with the classical advice of Hippocrates, "Ωφελέειν ου Βλάπτειν" — "Do good, not harm."

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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# Redirecting T Cells

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The pursuit of tumor-reactive T cells as a cancer therapy has continued unabated since the discovery of the graft-versus-leukemia effect in patients undergoing allogeneic hematopoietic stem-cell transplantation.[1] Some successes have been noted: the adoptive transfer of Epstein–Barr virus (EBV)–specific T cells can prevent and treat post-transplantation lymphomas,[2] and the adoptive transfer of in vitro activated and expanded autologous tumor-infiltrating lymphocytes after systemic depletion of lymphocytes induces durable complete remissions in some heavily pretreated patients with metastatic melanoma.[3] Therapy with tumor-infiltrating lymphocytes is difficult and expensive, and it has benefited only patients with melanoma. Redirecting T cells by gene transfer of T-cell receptors with predefined antigen specificity, which could overcome some of the problems with tumor-infiltrating lymphocytes, has not been very successful clinically as yet.

A parallel strategy has been to redirect T cells with chimeric antigen receptors, which include a targeting moiety, usually a single-chain Fv variable fragment from a monoclonal antibody, a transmembrane hinge region, and a signaling domain (typically the zeta chain from the T-cell signaling complex) (Fig. 1). Chimeric antigen receptors have theoretical advantages over other T-cell–based therapies. They use the patient's own cells, which avoids the risk of graft-versus-host disease. They can be created quickly, and the same chimeric antigen receptor can be used for multiple patients. Since chimeric antigen receptors recognize cell-surface molecules, they broaden the repertoire of potential targets to include cell-surface proteins, sugars, and lipids.[4] They also eliminate the requirement for restriction of the major histocompatibility complex (MHC) and overcome some of the evasion strategies used by tumors to escape recognition by the immune system (such as MHC loss or altered antigen presentation). First-generation chimeric antigen receptors had limited clinical activity, primarily because in vivo activation of the chimeric antigen receptor T cells induced only transient cell division and suboptimal cytokine production, which failed to produce prolonged T-cell expansion and sustained antitumor effects. These deficiencies were overcome by the addition of a costimulatory signaling domain in second-generation chimeric

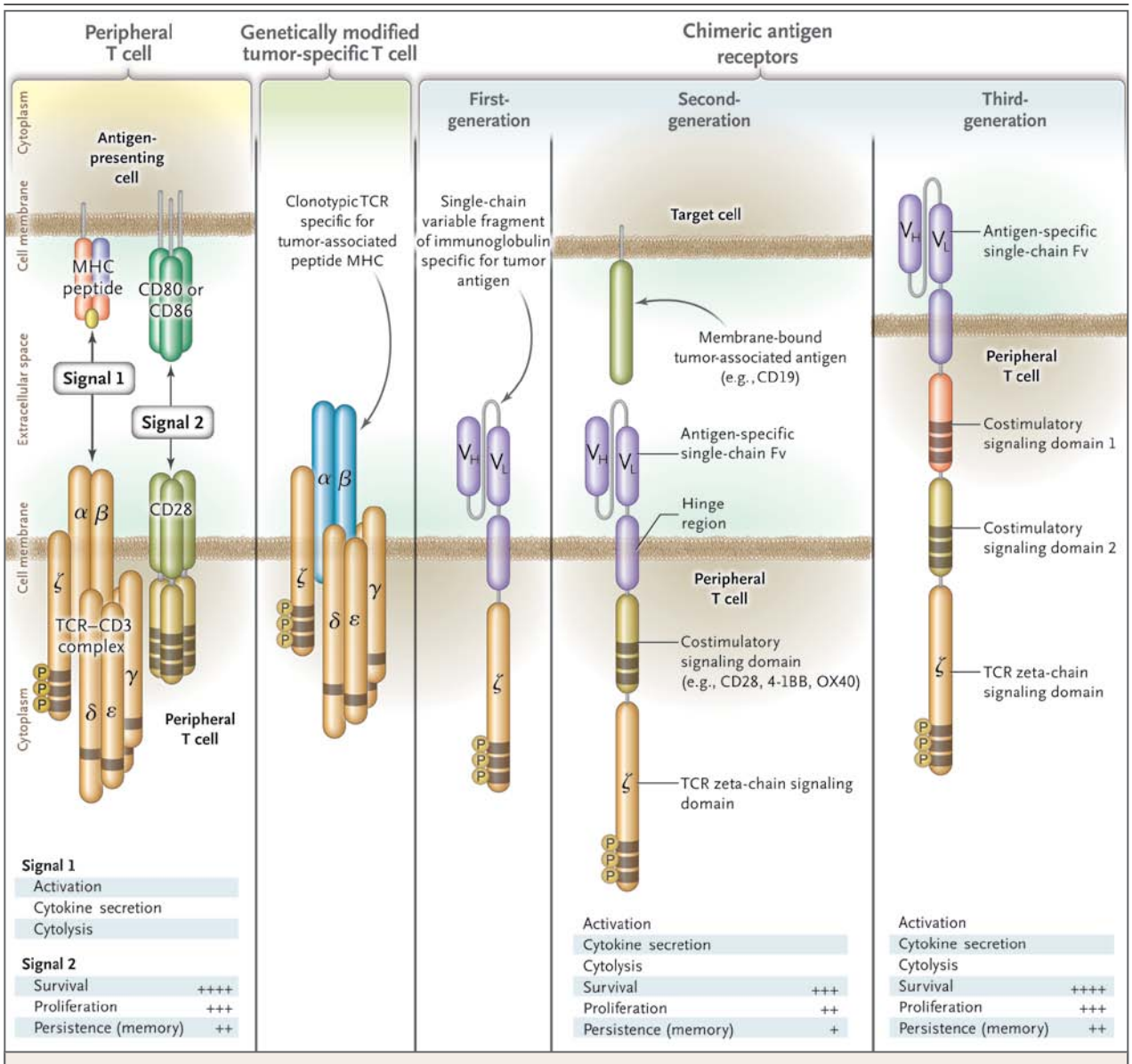


**Figure 1. T-Cell Activation.**

Optimal T-cell activation requires a minimum of two signals; signal 1 is delivered by the TCR–CD3 complex through interaction of the T-cell receptor (TCR) alpha and beta chains as they recognize peptide presented by a class I (CD8 T cells) or class II (CD4 T cells) major histocompatibility complex (MHC) molecule. Signal 2 is most commonly provided by the engagement of CD28 on the T cell with the costimulatory molecule CD80 or CD86 on the antigen-presenting cell. CD137 (4-1BB) and CD134 (OX40) also provide costimulation to T cells. The optimal combination of effector function, proliferation, and survival requires both signals. Delivery of signal 1 without costimulation, which often occurs for tumor-infiltrating lymphocytes and transgenic T cells encountering antigen on a solid tumor devoid of costimulatory molecules, leads to anergy and apoptosis, thereby limiting the antitumor response. The first-generation chimeric antigen receptors usually comprise a single-chain variable fragment of an antibody specific for tumor antigen linked to the transmembrane and intracellular signaling domain of CD3-zeta. Second-generation chimeric antigen receptors were developed to incorporate the signaling domain of a costimulatory molecule to improve T-cell activation and expansion. Third-generation chimeric antigen receptors include combinations of costimulatory domains. (Data are from Keith Bahjat, Ph.D.)

antigen receptors, which enhanced the proliferation, survival, and development of memory cells — features that appeared to be the hallmarks of successful therapy with EBV-specific T cells and tumor-infiltrating lymphocytes. The CD28 signaling domain has been used most commonly,

but CD137 (4-1BB) and CD134 (OX40) domains can also be effective.

In this issue of the *Journal*, Porter and colleagues[5] describe a heavily pretreated patient with chronic lymphocytic leukemia (CLL) who had a complete remission associated with the tumor lysis syndrome after adoptive immunotherapy with second-generation anti-CD19 chimeric antigen receptor–modified T cells. Four days after receiving chemotherapy with pentostatin and cyclophosphamide for depletion of lymphocytes, the patient received $1.42\times10^7$ transduced T cells over 3 days with no additional cytokines. Unselected peripheral-blood T cells were infected with a self-inactivating lentiviral vector carrying genes for the chimeric antigen receptor. These genes included the single-chain Fv from the hypervariable region of a human CD19-specific murine antibody, a hinge region, and human 4-1BB and CD3-zeta signaling domains. The tumor lysis syndrome was diagnosed 22 days after treatment and correlated temporally with the induction of high levels of cytokines (interferon-$\gamma$ and interleukin-6) and with an increase in the number of circulating chimeric antigen receptor–positive T cells to a level that was nearly 1000 times as high as the level detected the day after infusion. Eight months after therapy, chimeric antigen receptor–positive T cells persisted, and the patient had no evidence of disease on physical examination or on computed tomographic, flow-cytometric, or cytogenetic analysis.

The expansion, persistence, and development of the memory phenotype, not to mention antitumor effects, of these T cells were impressive.[6] The apparent superiority to CART19 T cells with only a CD28 domain[7] may be explained by the interaction of the CD28 ligands CD80 and CD86, which are present on CLL cells, with CD28 in addition to the 4-1BB signaling through the chimeric antigen receptor. In this respect, the chimeric antigen receptor may have functioned more like a third-generation construct that includes a combination of costimulatory domains. In addition to the tumor lysis syndrome, the patient had B-cell depletion and hypogammaglobulinemia. These conditions may not be major problems in patients with CLL, but in other tumor types, the persistence of activated T cells, memory T cells, or both could pose substantial problems. Both toxic effects to the target organ and also "on-target but off-organ" toxic effects have been observed because of unappreciated cross-reactive target antigens.[8] Toxicity may become more of a problem as more potent second- and third-generation chimeric antigen receptors are used in patients with different tumor types. Safety measures include the infusion of lower numbers of T cells, the use of immunosuppressive agents, and the introduction of an inducible "suicide signal" to kill the cells when they are creating mischief; a novel, nonimmunogenic, inducible caspase 9 suicide gene has been developed for this purpose.[9] The only deaths from toxic effects reported thus far have been acute and occurred within hours after administration of the gene-transfected cells,[7,10] — a situation in which the suicide strategy would not have had time to work.

Only with the more widespread clinical use of chimeric antigen–receptor T cells will we learn whether the results reported by Porter et al. reflect an authentic advance toward a clinically applicable and effective therapy or yet another promising lead that runs into a barrier that cannot be easily overcome.

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