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Double or nothing on cancer immunotherapy

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Abstract

Engineered T cells expressing two receptors distinguish malignant cells from healthy cells even in the absence of a tumor-specific antigen.

T cells modified to recognize tumors have the potential to be more effective than antibody drugs as cancer therapeutics^{1,2} —but only if they can be made to attack tumor cells specifically while sparing normal cells. Unfortunately, in this context immunologists have not been dealt a winning hand as few of the known tumor antigens are truly tumor specific. Most are expressed to some extent on healthy tissues, leading to side effects when they are recognized by engineered T cells. A report in this issue by Kloss *et al.*³ presents a T-cell immunotherapy strategy designed to avoid such collateral damage. The approach relies on combinatorial recognition of two antigens and careful titration of the strength of signaling through chimeric antigen receptors (CARs). If it can be translated to the clinic, it would enable tumor-specific, T cell-mediated targeting without the need for a tumor-specific antigen.

The idea of using T cells rather than antibodies to attack tumors is appealing for several reasons. In contrast to antibodies, naturally occurring or genetically engineered T lymphocytes are living cells with a massive capacity for proliferation and the ability to persist and form long-lasting memory lymphocytes. Perhaps more importantly, T cells are capable of detecting vanishingly small amounts of antigen and of responding by releasing tumor-killing cytokines and cytolytic mediators. In recent clinical trials for patients with metastatic melanoma as well as B-cell leukemias and lymphomas, durable clinical responses were observed after adoptive transfer of T cells transduced with T-cell receptors (TCRs) or CARs specific for antigens expressed on the tumor^{4–7}. TCRs and CARs recognize fundamentally different structures. Whereas TCRs recognize peptides that are usually derived from intracellular antigens and presented by molecules encoded by the major histocompatibility complex, CARs recognize ‘unprocessed’ molecules presented on cell surfaces. Both classes of receptors can be very sensitive and specific.

Yet the extreme sensitivity of T cells can also be a liability. It is becoming increasingly clear that expression of even a trace amount of antigen on normal tissues can result in ‘on-target,

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off-tumor' toxicities. In some cases, damage to normal tissues can be tolerated as the price of success. For example, treatment with T cells engineered to target CD19, an antigen expressed on some B-cell leukemia and lymphoma cells, can cause regression of tumor cells but also eradication of healthy B cells; the effects of B-cell loss can be countered to some degree by infusion of gamma globulin⁵⁻⁷. In other cases, damage to normal tissues can be devastating and even lethal. Such extreme, intolerable effects were observed in recent clinical trials using T cells. One trial used T cells that were engineered to express a CAR specific for ERBB2 in colon cancer⁸. The second used T cells designed to express a TCR specific for MAGE3 presented in the context of the HLA-A2 major histocompatibility complex molecule and was used to treat patients with melanoma, esophageal cancer and synovial sarcoma⁹.

Kloss *et al.*³ have devised a way to avoid or at least minimize unwanted off-tumor effects. Their approach enhances tumor specificity by targeting two antigens; although neither antigen is uniquely expressed on the tumor, co-expression of both antigens should be specific to the tumor (Fig. 1). The idea of dual targeting to increase T-cell reactivity to a tumor is not new. One recent study¹⁰ described T cells expressing a CAR specific for folate receptor 1 (FOLR1) and another CAR specific for ERBB2, and another group¹¹ combined a CAR specific for ERBB2 with a chimeric costimulatory receptor (CCR) specific for MUC1. However, neither report provided clear evidence that the dual-targeting approach protects normal tissues expressing either antigen from T cell-mediated attack.

Kloss *et al.*³ engineered human T cells in which the T-cell activation signaling function is carried out by a CAR specific for one antigen and the costimulation signaling function is executed by a CCR specific for the second antigen. Their first implementation of dual targeting was unsuccessful. T cells expressing a CD19-specific CAR and a prostate-specific membrane antigen (PSMA)-specific CCR attacked not only CD19⁺PSMA⁺ tumor cells but also CD19⁺PSMA⁻ tumor cells. Thus, the CD19-specific CAR (which mimics TCR signals) functioned independently of costimulation.

To address this problem, the authors hypothesized that a CAR with diminished activity would not activate T cells without the costimulatory signal from a co-expressed CCR. At the same time, they sought to test a more clinically relevant antigen pair and switched from PSMA and CD19 to PSMA and prostate stem cell antigen (PSCA), a pair of antigens highly expressed by some human prostate cancer cells. They searched for a weak CAR among a small panel of anti-PSCA single-chain antibody fragments (scFvs) and identified a construct whose estimated sensitivity was 1,000–10,000-fold lower than that of the most efficient scFv in the set. The weak scFv was converted into a CAR and expressed together with the anti-PSMA CCR in human T cells. The dual-targeting T cells eradicated PSCA⁺PSMA⁺ but not PSCA⁺PSMA⁻ tumor cells in mice, clearly discriminating between tumor cells expressing two antigens and tumor cells expressing only one antigen.

These results imply that even when no tumor-specific antigens are known, it may be possible to engineer T cells to attack malignant cells while avoiding healthy cells. This is important because the future success and impact of T cell-mediated cancer immunotherapy depends

on how many targetable tumor antigens can be identified. That said, many practical questions about the approach remain to be investigated.

First Kloss *et al.*³ judged the absence of ‘on-target, off-tumor’ effects in a somewhat contrived fashion. Off-tumor effects were gauged by quantifying the destruction of tumor cells—but not healthy tissues—expressing a single antigen. This experimental system may have been chosen because the authors sought to engineer human T cells to target human antigens. Thus, given the differences between human and mouse T cells, antigens and tissues, it would not have been informative to assess off-tumor effects of engineered human T cells on healthy mouse tissues. The dilemma of using human reagents in a humanized mouse versus the use of entirely murine experimental systems is one faced by every preclinical investigator. Nevertheless, the preclinical model used by Kloss *et al.*³ is suboptimal because, as normal tissue cells undergo less self-renewal than tumor cells, they are likely more susceptible to T cell-mediated killing. An experiment that used all mouse reagents could allow a stronger claim that engineered T cells can discriminate between normal and transformed tissues.

Another question relates to the practical process of selecting a CAR within the desired range of affinity for the tumor antigen. It may be difficult to design a CAR that works in most patients if its optimum affinity range is narrow or if the optimum affinity range varies widely depending on the amount of antigen expressed by tumor cells.

It is also not clear how many suitable antigen pairs will be available for a given tumor type and exactly how these antigen pairs should be selected. It is easy to imagine targeting pairs of antigens known to be expressed at higher levels on tumor cells than normal tissue, such as MUC1, ERBB2, FUT3, CD44, CD49f and EpCAM for breast cancer, FOLR1, CD44, CD133 and ERBB2 for ovarian cancer, and CEA, ERBB2 and MUC1 for colon cancer. Beyond this, appropriate targets could be selected using microarray databases; results obtained in this way could be confirmed by quantitative RT-PCR on newly isolated or archived tumor tissue samples. However, it would be essential to determine that a chosen antigen pair was not expressed on healthy cells, and for this a comprehensive database of RNAseq data covering all normal tissues would be invaluable.

The work of Kloss *et al.*³ stems from a keen appreciation of the importance of target identification in the field of tumor immunology. The practical utility of their approach will depend on the extent to which it protects normal tissues without hampering the desired anti-tumor T-cell response in a clinical setting. Data from clinical trials based on this strategy are eagerly awaited (but, to our knowledge, are not yet underway). In summary, although the work of selecting and safely targeting tumor antigen pairs will be challenging, the payoff may well be worth all the effort.

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Figure 1.

Strategy for designing T cells to attack tumor cells and spare normal tissues. In this scenario, antigens A and B are expressed on tumor cells, but neither are truly tumor specific. Antigen A is expressed on normal tissue 1, and antigen B is expressed on normal tissue 2; tumor cells uniquely express both antigens. T cells are transduced with a CAR that recognizes antigen A with low affinity and a CCR that recognizes antigen B with high affinity. When the engineered T cells encounter normal tissue 1 (a), they are not activated because the signal from the low-affinity CAR alone (dashed line) is not strong enough. When they encounter normal tissue 2 (b), they are also not activated because they do not receive a CAR signal (which mimics a TCR signal). The T cells become activated only when they receive both CAR and CCR signals upon encounter with tumor cells expressing both antigens A and B (c).