Improving the outcome of adoptive cell transfer by targeting tumor escape

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Adoptive T-cell transfer is among the most promising immunotherapies against cancer. To continue increasing the potential of this therapy, our studies focus on the inhibition of tumor recurrence. Recently, we have demonstrated several ways in which combination therapies involving multiple T-cell populations and immunostimulatory chemotherapy can enhance long-term survival.

Reports of positive results in adoptive cell transfer-based clinical trials are rapidly accumulating. Several groups have demonstrated objective responses to the therapy as well as striking complete responses in some patients.1,2 Such results feed a growing hope that this immunotherapy might become part of a cure for patients with few other treatment options.

As with most cancer therapies, incomplete responses and tumor recurrence are common following adoptive T-cell transfer.1,3 Many pathways have been revealed through which tumors can escape from a T-cell response, making it unsurprising that T-cell transfer alone is typically insufficient for tumor eradication. Nonetheless, some patients do achieve long-term complete tumor clearance following adoptive T-cell transfer,1 suggesting that this strategy has great potential. In order to consistently achieve optimal results, we must improve our ability to predict the most likely escape mechanisms of a specific tumor and rationally develop methods to block those pathways.

Two broad approaches through which adoptive cell transfer protocols might be improved are: first, modifications aimed at directly enhancing the efficacy of transferred T cells, and second, modifications aimed at blocking tumor escape from these T cells. Our study involved both of these approaches. Using a simple mouse model, we altered our established adoptive cell transfer protocol in two ways. First, we transferred two distinct cytotoxic T lymphocyte (CTL) populations rather than one. Second, we employed immunostimulatory chemotherapy when tumor escape was detected.

The T-cell preparations used in clinical trials vary widely in characteristics such as specificity, activation status, trafficking behavior and affinity. While, historically, most trials have used tumor-specific T cells isolated from the peripheral blood or tumor-infiltrating lymphocytes (TILs), new methods for introducing tumor-targeted TCRs and chimeric antigen receptors (CARs) into non-specific T cells have resulted in a great diversification of approaches.4,5 These re-engineered cells are designed to have high affinity and potency against tumor antigens, while TILs are generally of low responsiveness. Another important distinction is that TIL preparations include diverse T cells targeting multiple antigens, whereas re-engineering typically involves the transduction of an entire T-cell preparation with a single tumor-targeting TCR or CAR. Besides differences inherent to the T cells themselves, variations in ex vivo culture methods can also substantially alter the resulting T cell product. For example, instead of typical Type 1 (Th1) effector CTLs, specific alternative culture protocols preferentially generate central memory cells or type 17 (Th17) effector cells.6 CD4+ T cells are also highly variable in terms of phenotype and function, and some subsets appear to be as valuable for adoptive cell transfer as traditionally used CD8+ CTLs.6

Rather than attempting to define one ideal T-cell population, we reasoned that long-term survival could be improved by co-transferring divergent T-cell populations. Ovalbumin (OVA)-specific OT-I T cells are highly cytotoxic and effective as a single therapy against OVA-expressing B16 tumors. In contrast, gp100-specific Pmel T cells barely recognize OVA-expressing B16 cells in vitro and exert no therapeutic effects in vivo. In support of our hypothesis, we found that tumors are best controlled when both these T-cell populations are concomitantly transferred. Furthermore, when tumors did escape, antigen loss was less severe in mice receiving both OVA-specific and gp100-specific T cells than in mice administered T cells of a single specificity.7 If a similar phenomenon occurs in patients receiving monoclonal vs. polyclonal T-cell transfer, this finding raises an important argument for targeting multiple antigens. While it is already well-appreciated that multipronged therapies have the benefit of targeting tumor-cell variants that have already lost one or more antigens, the possibility that
when necessary, previously transferred antitumor T cells can be exploited against recurrent tumors. Chemotherapies such as cyclophosphamide are already available. Future work will undoubtedly identify other, more potent means of reactivating antitumor T cell populations. Perhaps, inhibitory receptor blockade\(^9\) or booster vaccination against tumor-associated antigens\(^10\) can provide the stimulation that is required for the complete eradication of recurrent tumors. The key will be to rationally design these rescue therapies based on what is known about target tumors and their likely mechanisms of immunoescape.\(^7\)

**Figure 1.** Adoptive T-cell transfer using clonal T cells often results in tumor escape following loss of the target antigen (left side), whereas the transfer of diverse T cells limits antigen loss-related problems (right side). When a tumor escapes without losing antigen expression, additional benefits can be obtained from previously transferred cells through the use of immunostimulatory rescue strategies and/or by counteracting the dominant immunoescape pathways activated by the tumor.

such therapies can also ameliorate therapy-induced antigen loss is equally significant. These observations lead directly to the second aspect of our study.

Supplemental therapies can greatly influence both the short-term and the long-term results of adoptive T-cell transfer. Chemotherapy and total body irradiation are administered prior to T-cell infusion to improve cell engraftment and, alongside, patient survival.\(^1\) In mice, cyclophosphamide has additionally been shown to modulate the function of regulatory T cells (Tregs) and dendritic cells.\(^8\) We hypothesized that a similar cyclophosphamide regimen could be used to re-stimulate the antitumor response long after T-cell transfer, when tumors begin to escape. In testing this idea, we found that low-dose cyclophosphamide slowed the growth of escape tumors in mice that had previously been treated with adoptive T-cell transfer, but it had no effects in mice that had never received antitumor T cells. Supporting the notion that cyclophosphamide was inducing the reactivation of T cells, cyclophosphamide increased the responsiveness of Pmel T cells against both peptide antigen and tumor cells in vitro.\(^7\) While our therapy did not result in the elimination of recurrent tumors, it did demonstrate that T-cell reactivation can be a valuable option for patients with recurrent tumors.

**Figure 1** depicts our two-steps approach for enhancing long-term survival in adoptive T-cell transfer. By starting with a diverse T-cell population, escape through antigen loss can be limited. In this way, when necessary, previously transferred antitumor T cells can be exploited against recurrent tumors. Chemotherapies such as cyclophosphamide are already available. Future work will undoubtedly identify other, more potent means of reactivating antitumor T cell populations. Perhaps, inhibitory receptor blockade\(^9\) or booster vaccination against tumor-associated antigens\(^10\) can provide the stimulation that is required for the complete eradication of recurrent tumors. The key will be to rationally design these rescue therapies based on what is known about target tumors and their likely mechanisms of immunoescape.

**Notice of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
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