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CD137 as a Biomarker for Tumor-Reactive T Cells: Finding Gold in the Desert

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Although human cancer is often surrounded by immune cells, only a small number are tumor-reactive T cells that recognize the tumor antigens and are able to eliminate the cancer cells. Ye and colleagues now found that many of these tumor-reactive T cells are marked by expressing CD137, a T-cell costimulatory receptor. *Clin Cancer Res; 20(1); 3–5. ©2013 AACR.*

In this issue of *Clinical Cancer Research*, Ye and colleagues use CD137 as a marker to successfully enrich and expand tumor-specific T cells from cancer tissues for adaptive immunotherapy (1). Adoptive cell therapy (ACT) has been very effective in the clinical treatment of advanced melanoma. It involves isolation, *ex vivo* activation, and expansion of autologous cancer-specific T cells from patients with cancer to large numbers, which will eventually be transferred back to the patients to combat cancer. Approaches to ACT using autologous cells genetically engineered to express the antitumor T-cell receptor are under vigorous development (2). The immunogenicity of melanomas makes them feasible for ACT. Tumor-reactive T cells can be derived from the blood or the surgically resected tumors of patients with melanoma, though the latter environment is more enriched in tumor-reactive T cells. The first clinical trial of adoptive T-cell transfer using tumor-infiltrating lymphocytes (TIL) demonstrated a significant objective response among patients with metastatic melanoma (3). Later on, a lymphodepleting regimen was given to patients before cell transfer to improve the persistence of the transferred cells. In addition, efforts have been made to shorten the culture period *in vitro*, which is believed to improve the quality of T cells for transfer. A recent clinical trial from the National Cancer Institute indicates that the transfer of short-term cultured TILs together with lymphodepleting chemotherapy can trigger tumor regression in up to 50% of metastatic melanoma with manageable toxicity (4).

A prerequisite to ACT is the isolation of a sufficient number of tumor-specific T cells from the patient. TILs are a mixture of different T-cell subsets. TILs are composed of T cells with an effector memory phenotype, and many of them are anergic or exhibit an exhausted phenotype originally described in chronic viral infection. Significant numbers of immunosuppressive cells, including both $Foxp3^+$ and $Foxp3^-$ regulatory cells, are also observed in TILs from most types of cancer. Thus, the rationale for ACT is to identify the ideal T-cell subtype to maximize the antitumor immune response while eliminating suppressor T cells from TILs. The identification of cancer-associated antigens spurred interest in the derivation and transfer of $CD8^+$ T-cell clones for cancer therapy in the late 1990s. However, cases of cancer recurrence caused by antigen loss have been documented, supporting an argument against the use of T-cell clones with limited epitope recognition. In addition, tumor antigens for many cancers other than melanoma are still not well characterized. Therefore, it is valuable to look for alternative biomarkers for tumor-reactive T cells without the identity of tumor antigens.

CD137, also called 4-1BB, is a member of the TNF receptor superfamily with T-cell costimulatory functions (5). Signaling through CD137 by its natural ligand, CD137L, or agonistic antibody promotes the expansion of T cells, sustains their survival, and enhances their cytolytic effector functions. Naïve T cells do not express CD137; however, upon stimulation, activated T cells transiently express a high level of CD137, which disappears rapidly. Memory T cells respond to CD137 signal directly to expand. However, they express minimal level of surface CD137, and local signals in the bone marrow and liver contribute to CD137 upregulation on memory T cells (6). Interestingly, CD137 was seen to be selectively upregulated upon allo-antigen stimulation, and CD137 depletion was able to remove the allo-reactive T cells during hematopoietic transplantation (7). Similarly, CD137 expression could be used to isolate the full repertoire of $CD8^+$ T cells in response to antigen without the knowledge of epitope specificities (8). Thus, the expression of CD137 on T cells is tightly regulated and is extremely sensitive in response to antigen stimulation.

In this study, Ye and colleagues made an effort to see whether CD137 could be used as a marker to selectively isolate tumor-specific T cells from TILs (Fig. 1). They found that CD137 is preferentially expressed on T cells directly

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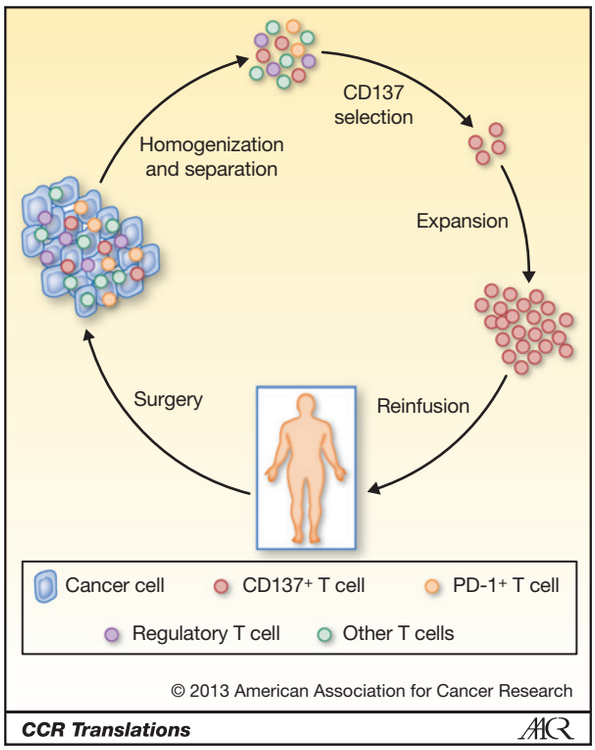

Figure 1. Isolating CD137$^+$ T cells from cancer tissues for ACT. CD137 is selectively expressed on tumor-reactive T cells in TILs. After the enrichment of these CD137$^+$ cells from single-cell suspension of cancer tissues, these cells undergo a rapid expansion process to generate enough T cells to be infused back into the same patient.

isolated from tumor or ascites of human ovarian cancer. Overnight incubation with autologous tumor cells significantly increased the frequency of CD137$^+$ T cells in TILs, which seems to be antigen-specific. The antigen-dependent CD137 upregulation was further confirmed when the author used T-cell lines with known antigen specificity. When T cells were isolated and separated from tumors on the basis of CD137 expression, the CD137$^+$, but not the CD137$^-$ T cells were able to respond to autologous tumor-cell stimulation. More importantly, when tumor cells and human T cells are cotransferred into immunodeficient mice, only CD137$^+$ T cells could inhibit tumor cell growth *in vivo*.

Many T-cell coreceptors associated with T-cell exhaustion are expressed on TILs, including PD-1, TIM-3, and LAG3 (9). Recently, PD-1 has been reported to be used as a biomarker to isolate tumor-reactive T cells (10). However, the quality of those T cells has not been fully investigated. In this study, Ye and colleagues sorted out different T-cell subsets from TILs on the basis of the expression of CD137 and PD-1, and compared their response with tumor antigen stimulation. CD137$^+$ TILs produced significantly more IFN-γ than PD-1$^+$ cells upon tumor stimulation. As CD137 expression on T cells is transient in response to stimulus, CD137$^+$ TILs represent a group of newly recruited T cells. In contrast, PD-1$^+$ T cells are exhausted resident TILs, which constantly expose to tumor antigen. In all, CD137 represents a superior biomarker for tumor-reactive T cells with better quality.

Overall, the study by Ye and colleagues described a new way of isolating human TILs effectively for ACT. With this approach, the broad diversity of antigen receptors (T-cell receptors) of TILs is preserved, which should help to prevent cancer cells with mutated antigen from escaping. In addition, this method is applicable to both melanoma and ovarian cancer without known antigen specificity. As each round of stimulation drives the cells further toward terminal differentiation or exhaustion, this method could enrich enough reactive cells after fewer divisions, which could have a direct impact on the quality of selected cells. Finally, the selective upregulation of CD137 receptor on tumor-reactive T cells in human cancer in this study also validates CD137 monoclonal antibody (mAb)targeting cancer immunotherapy. Consistently, the antitumor effect of CD137 agonist mAb has been seen in various types of mouse tumor models (11). An early trial of CD137 mAb in cancer showed very promising clinical results, whereas severe hepatitis was seen in patients (12). Therefore, it will be interesting to see whether agonist CD137 mAb could also enhance TIL functions during an *in vitro* selection process so that we can double the benefit.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: L. Chen
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Chen
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Chen

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