

## Zeroing in on Tumor-Reactive TILs

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Adoptive cell transfer of tumor-specific T cells provides an effective strategy for cancer immunotherapy. An article in this issue provides a novel approach to refine this technology by identifying tumor-reactive T cells based on frequency and PD-1 expression. *Cancer Immunol Res*; 4(9); 719. ©2016 AACR.

See article by Pasetto et al., p. 734.

The infiltration of CD8<sup>+</sup> T cells into tumors correlates with an improved patient prognosis in multiple tumor sites (1). This suggests that a population of tumor-specific T cells provides natural immune surveillance and can contribute to controlling tumor growth. The Rosenberg team has developed a successful adoptive T-cell therapy program based on tumor-infiltrating lymphocytes (TIL) and have demonstrated remarkable durable responses in metastatic melanoma (2). However, questions remain as to whether TILs can be improved, or whether other insights can be made from the TIL population. For example, it is not clear what proportion of T cells in TILs are truly tumor specific, or if many of the infiltrating T cells are bystander non-tumor-reactive T cells that have trafficked into the tumor because they were receptive to certain inflammatory cues.

Here, Pasetto and colleagues have identified tumor-reactive TILs using TCR frequency and expression of PD-1 as a marker. Blocking PD-1's engagement with its ligand is an effective way to reactivate exhausted cells (3) and enhance effector function in responding T cells (4). In this study, they defined rearranged TCR $\alpha/\beta$  heterodimers from CD8<sup>+</sup> PD-1<sup>+</sup> TILs, then cloned and transduced the TCR heterodimer into peripheral blood lymphocytes (PBL). The TCR-transduced PBLs were tested for tumor reactivity by coculturing them either with autologous tumor cell lines or with their own antigen-presenting cells transduced with (i) tandem minigene constructs encoding defined mutated antigens unique to each patient, or (ii) vectors expressing full-length common self-antigens (MART-1, gp100,

tyrosinase, NY-ESO-1, MAGEA3, and SSX2). Reactivity to tumor antigens and subsequent activation was determined by the upregulation of 41BB expression. Pasetto and colleagues found that the most frequent T-cell clone in 7 of 11 patients was specific for their tumors. From the top two most frequent T-cell clones, 9 of 11 patients had at least one tumor-specific TCR identified. An important feature of this study is that the TILs used were straight from the tumor, as opposed to *in vitro*-expanded TIL cultures.

The authors also outline some of the drawbacks of this approach, including the uncertainties associated with assigning TCR $\alpha/\beta$  pairs. If a PBL transduced with a TCR $\alpha/\beta$  heterodimer does not show reactivity, one cannot conclude that it originated from a TCR that does not recognize tumor antigens because it may not actually represent a "valid" TCR heterodimer. In addition, it is unclear whether or not the transduced  $\alpha/\beta$  TCR pair was expressed at similar levels as the endogenous TCR, and this may be required to reach a signaling threshold sufficient for T-cell activation. However, these points would result in difficulty defining tumor-antigen reactivity, but do not apply to situations in which the TCR specificity was defined.

The findings from this study have the potential to positively affect the field in several ways. (i) Using this approach, tumor-specific T cells can be generated and cloned without prior identification of target antigens. Therefore, it is possible to rapidly clone one or more tumor-reactive TCRs for TCR transduction into PBLs for therapy. (ii) This approach also provides a strategy to select a polyclonal repertoire of tumor-reactive T cells. It is possible that improved responses to TIL-based therapies would be observed if the TIL product is enriched for tumor reactivity. Therefore, unique protocols may be developed using the ideas presented here to improve cell therapy-based strategies for immunotherapy.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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