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TCR Repertoire Changes during TIL Expansion: Clonal Selection or Drifting?

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SUMMARY

T-cell receptor β analysis can be exploited to monitor changes in the clonotypic composition of tumor-infiltrating lymphocyte (TIL) during *ex vivo* expansion in IL2. Oligoclonal TILs are often outgrown by infrequent clonotypes with greater proliferative capacity, and this could impact the antitumor reactivity of the expanded TILs.

See related article by Poschke et al., p. 4289

In this issue of *Clinical Cancer Research*, Poschke and colleagues used T-cell receptor β (TCRβ) deep sequencing to investigate the TCRβ repertoire dynamics during tumor-infiltrating lymphocyte (TIL) culture (1).

Ex vivo expansion of TIL relies on the nonspecific expansion of lymphocytes present in tumor single-cell suspensions or tumor fragments in high dose IL2 for 2–4 weeks. This initial TIL growth is followed by a large-scale expansion for patient treatment, which entails nonspecific stimulation via anti-CD3 in the presence of irradiated feeders and IL2 for 14 days, also known as rapid expansion protocol (REP). Expanded TILs are polyclonal, they comprise a mixture of $CD8^+$ and $CD4^+$ T cells, and are variably enriched in tumor-reactive lymphocytes. TILs can target a variety of antigens including tissue-specific antigens, self-antigens, cancer germline antigens, and mutated tumor neoantigens. Importantly, despite small variations in TIL generation, multiple institutions have shown that a single infusion of *ex vivo* expanded TILs following a nonmyeloablative lymphodepleting conditioning regimen and exogenous IL2 can mediate objective tumor regression in approximately 50% of patients with metastatic melanoma (2). Hence, adoptive transfer of TIL represents a potent therapy, and ongoing research focuses on extending the antitumor efficacy observed to patients that are refractory to immune checkpoint inhibitors or patients with other solid cancers.

Retrospective correlative studies support that $CD8^+$ lymphocytes are the primary drivers of tumor rejection in patients with metastatic melanoma treated with TIL (3), suggesting that enrichment of tumor-reactive lymphocytes could potentiate clinical efficacy. However, the rare availability of tumor targets has hindered the prospective selection of TIL based on autologous tumor recognition. Consequently, patients are treated with largely uncharacterized TIL products containing an unknown frequency of tumor-reactive lymphocytes. TCRβ deep sequencing can be used to monitor T-cell responses to a given immune challenge without *a priori* knowledge of the specific epitope targeted. This technique has provided critical insights to identify tumor-resident T-cell subpopulations enriched in tumor-reactive lymphocytes without the need to screen T cells for tumor recognition. TCRβ deep sequencing of lymphocytes infiltrating melanoma, as well as other tumors, has revealed that $CD8^+$ cells expressing markers of T-cell dysfunction such as PD-1 are clonally expanded (4). In addition, the most frequent TCR clonotypes within the $CD8^+PD\text{-}1^+$ melanoma-resident population, which were also often among the most prevalent TCRs in the bulk TIL population, were reported to frequently recognize tumor or neoantigens (5). However, the fate of the TCRβ repertoire over the course of TIL culture remains unexplored.

The authors of this article show that the TCRβ repertoire of T cells infiltrating pancreatic ductal adenocarcinoma (PDA) and melanoma is oligoclonal compared with peripheral blood, suggesting antigen-specific clonal expansion. More importantly, they expanded PDA, as well as melanoma TILs and demonstrated that *ex vivo* expansion of TILs in IL2 can cause major changes in the clonotypic composition of the TIL originally present at the tumor (**Fig. 1**). These changes were characterized by a reduction in TCRβ diversity, paralleled by an increase in clonality. Remarkably, the clonotypes that were most prevalent originally were often overgrown by rare clonotypes. Although, this observation appears to be more prominent in PDA TIL ($n = 25$) where the average number of the top 25 most frequent TCRβ clonotypes maintained after culture was 1.8, the decline of prevalent TCRβ clonotypes was also commonly observed following melanoma TIL expansion ($n = 20$). Interestingly, most TCRβ changes tend to occur during the first phase of TIL expansion, not the REP.

The observed changes in TCRβ composition raised the question of whether they could result from clonal selection, influenced by T-cell intrinsic factors of the original TIL population, or through clonal drifting happening by chance. To explore the former, the authors investigated whether specific phenotypic traits impacted the proliferative capacity of >2,000 individual $CD3^+$ lymphocytes derived from eight different PDA tumors using single-cell index sorting. They found that expression of CD8, PD-1, and effector memory phenotypic markers was negatively associated with their proliferative capacity, which supports the T-cell dysfunction previously attributed to $CD8^+PD\text{-}1^+$ TIL. Importantly, the $CD8^+PD\text{-}1^+$–resident TIL population is typically more oligoclonal than the $CD8^+PD\text{-}1^-$ subset. This offers a plausible explanation for the decline in the oligoclonal TCRβ TIL observed and suggests that TIL expansion exerts a pressure that results in the selection of rare clonotypes with greater proliferative capacity that are likely less dysfunctional.

Although clonal selection may contribute to shape the TCRβ repertoire during *ex vivo* culture of TIL, spatial tumor heterogeneity appeared to impact the most. The authors report that the loss of clonally expanded TCRβ clonotypes differed greatly among TIL expanded from nonadjacent tumor fragments. While a considerable overlap was found in the initial TIL TCRβ repertoire among spatially

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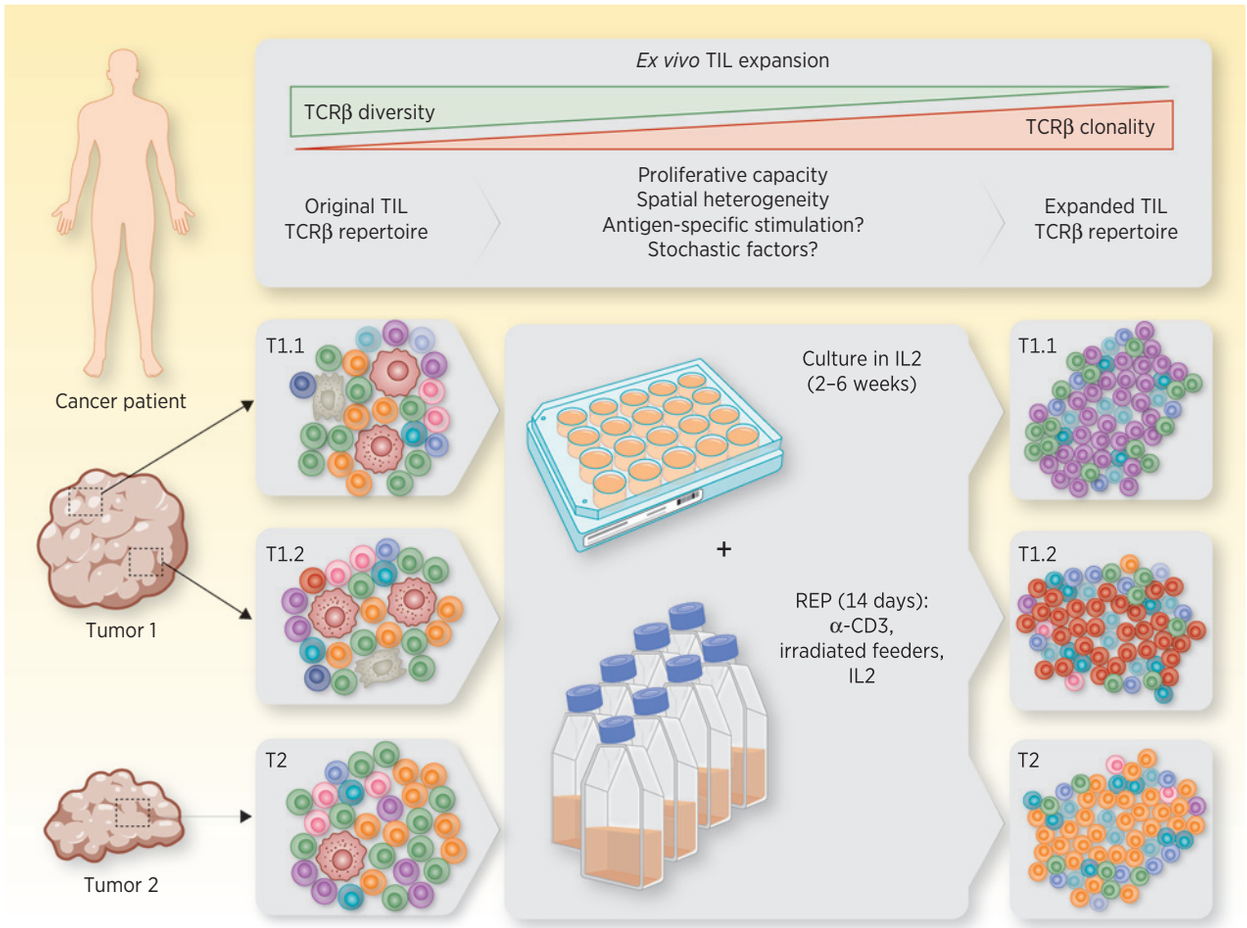

Figure 1.
Impact of *ex vivo* culture on the TCRβ repertoire of TIL. The TIL TCRβ repertoire of spatially distant fragments within the same or a different tumor deposit from one patient with metastatic cancer is depicted before and after *ex vivo* expansion. Each unique TCRβ clonotype is represented in a different color. Cultures initially contained both T cells (round small cells) as well as other cell types including tumor cells, macrophages, and others that are not represented. A summary of the characteristic changes observed in the diversity and clonality of the TCRβ repertoire following TIL culture and the potential factors that could influence these changes are shown (top). Adapted from an image created with BioRender.com.

distant fragments within the same or different tumor deposits, this overlap was particularly more evident when considering the most prevalent clonotypes. The less frequent clonotypes, on the other hand, were sometimes uniquely enriched or detected in a specific region of the tumor or one of several tumor deposits studied. In view that rare TCRβ clonotypes often overgrow during TIL culture, it is tempting to hypothesize that culturing TIL from independent tumor fragments could act as a founder effect, where a reduced and variable sample size and the presence of uniquely enriched clonotypes in one region, but not in others, could account for the clonal drift observed in the expanded TIL.

As the authors speculate, the loss of the prevalent TCRβ clonotypes observed could indirectly suggest a decrease in tumor reactivity by the expanded TIL. This idea is supported by the data demonstrating that clonally expanded TCRs in melanoma tumors are often tumor reactive (5). Moreover, the $CD8^{+}PD\text{-}1^{+}$ lymphocytes, which appear to be in proliferative disadvantage, are frequently enriched in tumor-reactive lymphocytes in melanoma as well as other tumors (4). Consistent with this hypothesis, several recent reports have suggested a gradual decrease in the frequency of neoantigen-reactive lymphocytes during TIL culture from gastrointestinal cancers. If this was indeed the case, TCRβ deep sequencing of parallel TIL cultures expanded from nonadjacent tumor fragments or from different tumors could emerge as a valuable tool to select those TILs that best preserve the initial TIL TCRβ repertoire. Alternatively, the selection of PD-1 or 4-1BB TIL subsets enriched for tumor recognition or the isolation of neoantigen-specific TCRs and the generation of personalized gene-engineered T-cell therapies could overcome a potential decline in tumor recognition during TIL expansion.

And yet, the true impact of the loss of the most prevalent clonotypes in the antitumor reactivity of the expanded TIL and TIL therapy will require further investigation, because the antitumor specificity of the waning and newly emerging TCR clonotypes was not evaluated. While clonal selection and drifting are likely to influence the expanded TCRβ repertoire, additional factors could impact, which have not yet been explored. Among these, the interaction of T cells with tumor cells, particularly during the first phase of TIL culture, could favor the preferential expansion of tumor-specific lymphocytes, even if initially

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rare. Selection of the "fitter" clonotypes could even be desirable, if the newly emerging clonotypes were capable of recognizing tumor.

Collectively, while some fluctuation in the frequency of the original TCRβ repertoire during TIL culture could be anticipated, the often dramatic changes observed both in PDA- and melanoma-derived TIL are striking, and the impact of these findings in the efficacy of TIL therapy warrants further investigation.

Disclosure of Potential Conflicts of Interest

A. Gros reports grants from Novartis, VCN Biosciences, and Merck KGaA, and personal fees (consultant) from Roche, Achilles Therapeutics, Neon Therapeutics, Genentech, and Pact Pharma outside the submitted work, as well as a patent for E-059-2013/0 licensed and with royalties paid from Intima Bioscience Inc., Intellia Therapeutics, Inc., Tailored Therapeutics, LLC, Cellular Biomedicine Group, Inc., and Geneius Biotechnology, Inc., a patent for E-085-2013/0 licensed and with royalties paid from Intima Bioscience Inc., Intellia Therapeutics, Inc., and Geneius Biotechnology, Inc., and a patent for E-149-2015/0 licensed and with royalties paid from Intima Bioscience Inc., Intellia Therapeutics, Inc., and Tailored Therapeutics, LLC. No potential conflicts of interest were disclosed by the other author.

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