Gamma delta T-cell response to cellular stress signals expressed by tumor cells makes them promising candidates for cancer immunotherapy. The proof of concept for clinical scale propagation of polyclonal γδ T-cell lines with efficient in vitro and in vivo response against cancer is an important step in this direction. Clin Cancer Res; 20(22); 1–3. ©2014 AACR.

In this issue of Clinical Cancer Research, the articles by Deniger and colleagues (1) and by Fisher and colleagues (2) report on a novel process for scaling up in vitro expansion of polyclonal γδ T-cell lines with killing activity against cancer cells for clinical use. T-cell therapy to treat cancer has been the focus of much interest in the past years and led to the recent success of chimeric antigen receptors–expressing T cells (reviewed in ref. 3). This interest mainly concentrated on βγ T cells whose mode of antigen recognition, and thus of activation, has been fully resolved. Because of their ability to recognize different stress signals provided by tumor cells, γδ T cells also hold an alternative promise in cancer therapy. So far, much attention has been given to the predominant subset of circulating γδ T cells, the Vγ9Vδ2 T cells, which are strongly activated by nonpeptide phosphorylated metabolites of isoprenoid biosynthesis pathway (called phosphoantigens). Phosphoantigens are produced at high levels by tumor cells and have been successfully used to generate clinical scale quantities of Vγ9Vδ2 T cells in vitro and to expand them in vivo in active vaccination-type trials. Although remarkably safe and superior to current second-line therapies in some settings, Vγ9Vδ2 T-cell–based trials in patients with cancer provided mixed results, suggesting room for improvement in the therapeutic use of γδ T cells (4).

Besides Vγ9Vδ2 T cells, all the other γδ T cells (collectively called Vδ2reg γδ T cells) populate many epithelial tissues where they represent an important component of intraepithelial lymphocytes, so likely the main subset of human γδ T cells in the whole body, and an important first-line defense against diverse host assaults. Despite increasing evidence of their role in tumor surveillance, Vδ2reg γδ T cells have been largely neglected in cancer cell therapy because of the limited knowledge about the antigens they recognize, which have restricted their specific and large-scale expansion for clinical purposes. One method to grow large quantities of whole γδ T cells was developed by Lopez and colleagues (5) but has not yet been used in cell therapy trials.

Deniger and colleagues (1) and Fisher and colleagues (2) used artificial antigen-presenting cells (aAPC) for clinical scale in vitro expansion of polyclonal γδ T-cell lines comprising both Vδ2pos and Vδ2neg γδ T cells (Fig. 1). They took advantage of aAPCs available as clinical-grade reagents and already used to manufacture high numbers of βγ T cells and NK cells for clinical trials at the MD Anderson Cancer Center (6). These aAPCs are based on K562 tumor cells genetically modified to express CD64, CD86, CD137L, and a membrane-bound form of IL15. Culturing pure γδ T cells sorted from either peripheral blood mononuclear cells (PBMC) or umbilical cord blood with aAPCs, in combination with exogenous IL2 and IL21, led to a remarkably high expansion of γδ T cells, providing clinical-scale amount of cells (>10⁹ from PBMCs and >10¹¹ from cord blood). Although ex vivo stimulation of Vγ9Vδ2 T cells from patients with cancer is often limited using phosphoantigens, Fisher and colleagues (2) also obtained high expansion rate of γδ T cells from children with neuroblastoma with this aAPC-based protocol. Using either mRNA quantification through nonenzymatic digital multiplex assay (i) or next-generation sequencing (ii), both teams performed in-depth γδ T-cell receptor (TCR) TCR repertoire analysis of the generated γδ T-cell lines. Although not exactly reflecting the initial composition of γδ T cells, the repertoire of expanded T cells was polyclonal with expression of all functional Vδ and Vγ chains.

It is not yet clear which are the activation signals underpinning this large expansion of polyclonal γδ T cells in this process. Proliferation was dependent on CD137L, IL2, and IL21, but the involvement of TCR signaling has not been tested. Although it is hard to conceive that aAPCs express the full array of TCR ligands for all expanded γδ T cells, engagement of TCR is suggested by the low TCR expression on generated Vδ2pos T cells and by the importance of CD137L for the expansion despite the absence of CD137 expression by γδ T cells prior expansion. Fisher and colleagues who coated B1 anti-γδTCR antibody on aAPCs showed it did not have a major role in γδ T-cell expansion even if this led to a better representation of Vδ2neg subsets. Gamma delta T cells...
Autologous settings implying expansion from cells of patients with cancer are possible as shown by efficient expansion of γδ T cells from patients with neuroblastoma. Alternatively, as γδ T cells are not restricted by classical MHC and have no alloreactivity (8), infusion of γδ T cells generated from unrelated healthy donor is feasible with limited risk for GVHD, as recently done for haploidentical Vδ2 pos expanded cells (9). Expression of CD16 on Vδ2 pos cells also paves the way for combined therapy with therapeutic antibodies. Finally, the widely reported involvement of γδ T cells in the protection against infections might provide collateral benefit of their injection in patients with cancer susceptible to infectious diseases because of chemotheraphy.

Clinical trials using aAPC-generated γδ T cells should also shed light on still pending but interesting issues: Are these cells able to migrate into the tumors? Can they expand in vivo after infusion? Is quantity of infused cells a major issue or could therapeutic potential be improved by increasing their specificity toward defined tumors or defined antigens? Altogether with the previously reported association between Vδ1 subset expansion and complete response in hematopoietic stem cell transplantation patients with leukemia (10), and the mitigated but encouraging results obtained from Vγ9Vδ2 pos cells able to migrate into the tumors, and capacity to reduce tumor biomass and increase survival in an ovarian cancer xenograft model.

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No potential conflicts of interest were disclosed.

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