Promising Cell-Based Immunotherapy Using Gamma-Delta T Cells: Together Is Better

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Running Title: Cancer Immunotherapy Using Polyclonal Gamma-Delta T Cells

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γδ T cell response to cellular stress signals expressed by tumor cells make 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.

In this issue of *Clinical Cancer Research*, two papers by Cooper and colleagues (1) and by Anderson 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 (CAR)-expressing T cells (reviewed in (3)). This interest mainly concentrated on αβ 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 cancer patients provided mixed results suggesting room for improvement in the therapeutical use of γδ T cells (4).

Besides Vγ9Vδ2 T cells, all the other γδ T cells (collectively called Vδ2<sup>neg</sup> γδ T cells) populate many epithelial tissues where they represent an important component of intra-epithelial 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, Vδ2neg 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 et al (5) but has not yet been used in cell therapy trials.

Cooper and colleagues and Anderson and colleagues 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 aAPC available as clinical grade reagents and already used to manufacture high numbers of αβ T cells and NK cells for clinical trials at MD Anderson Cancer Center (6). These aAPC 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 PBMC or umbilical cord blood with aAPC, in combination with exogenous IL2 and IL21, led to a remarkably high expansion of γδ T cells, providing clinical-scale amount of cells (>10^9 from PBMC and >10^11 from cord blood). While ex vivo stimulation of Vγ9Vδ2 T cells from cancer patients is often limited using phosphoantigens, Fisher et al also obtained high expansion rate of γδ T cells from children with neuroblastoma with this aAPC-based protocol. Using either mRNA quantification through non-enzymatic digital multiplex assay (1) or next generation sequencing (2), both teams performed in depth γδTCR 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 aAPC 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 et al who coated B1 anti-γδ TCR antibody on aAPC showed it did not have a major role in γδ T cell expansion even if this led to a better representation of Vδ2neg subsets. γδ T cells have been shown to express HLA-I inhibitory receptors (7), so the absence of HLA-I expression by K562 as well as expression of activatory molecules such as NKG2D-ligands may also play critical role in γδ T cell activation.

Most interestingly, aAPC-propagated γδ T cells displayed efficient response against cancer cells, with an equivalent contribution of Vδ2pos and Vδ2neg γδ T cells. A strikingly broad killing capacity against tumor cell lines from acute or chronic leukemia, colon, pancreatic and ovarian cancers was developed whereas no killing of autologous or allogeneic normal B cells ensued. This panel of targets was extended to neuroblastoma cell lines in Fisher et al paper which also reports on an enhanced killing in the presence of an anti-ganglioside G2 mAb consistent with the expression of CD16 on a significant proportion of aAPC expanded Vδ2pos cells. Tumor killing was accompanied by IFNγ production which could be an additional mean for γδ T cells to control tumours.

To test whether polyclonal γδ T cells are able to kill tumors in vivo, Deniger and colleagues used an ovarian cancer xenograft model in immunodeficient mice and in vivo bioluminescence imaging. The bioburdens of established tumors were considerably reduced 72 days after injection of polyclonal or separate subsets of expanded γδ T cells, resulting in an increased long-term survival of mice.
Further investigations are needed to understand how aAPC-generated γδ T cells recognize and kill cancer cells. Combined blocking of NKG2D, DNAM1 and the TCR decreased cancer cell killing, suggesting a complex multimolecular-based cancer cell recognition by γδ T cells. Antigen recognition by polyclonal γδ TCRs on such a broad spectrum of cancer cell raises the question of their frequency and specificity, opening perspectives for identification of new γδ TCR ligands.

These results have strong potential for translation in clinical practice. The aAPC-mediated expansion procedure and reagents have already been approved for clinical use. Autologous settings implying expansion from cancer patient's cells are possible as shown by efficient expansion of γδ T cells from neuroblastoma patients. 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 Vδ2pos expanded cells (9). Expression of CD16 on Vδ2pos 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 cancer patients susceptible to infectious diseases because of chemotherapy.

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 tumours? 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 tumours or defined antigens? Altogether with the previously reported association between Vδ1 subset expansion and complete response in HSCT patients with leukemia (10), and the mitigated but encouraging results obtained from Vγ9Vδ2 T cell-based cancer therapy, the infusion of polyclonal γδ T
cells with broad cancer specificity deserves deep attention as a valuable additional tool in the therapeutical arsenal against cancer.

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**References**


Figure 1. Activation of γδ T cells purified from PBMC or cord blood with aAPC induces clinical-grade expansion of polyclonal γδ T cell lines with broad specificity toward cancer cells, and capacity to reduce tumour biomass and increase survival in an ovarian cancer xenograft model.
Killing of tumor cells in vitro

Elimination of xenograft tumors

> $10^8$ cells

IL2

IL21

CD86

CD137L

IL15

aAPC (K562) K562 K562

V\textsubscript{D}1 cell

V\textsubscript{D}3 cell

V\textsubscript{D}5 cell

V\textsubscript{D}8 cell

V\textsubscript{D}7 cell

V\textsubscript{D}4 cell

V\textsubscript{D}2 cell

Figure 1: