Behind the Paper

Unlocking the potential of allogeneic Vδ2 T cells for ovarian cancer therapy through CD16 biomarker selection and CAR/IL-15 engineering

We employed CD16 as a biomarker to identify and select Vδ2 T cell PBMC donors. CD16 high Vδ2 T cells were then engineered with a Mesothelin-targeted chimeric antigen receptor and soluble IL-15 for the treatment of ovarian cancers

Published Nov 14, 2023

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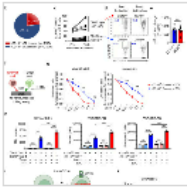

Nature

Unlocking the potential of allogeneic Vδ2 T cells for ovarian cancer...

Vγ9Vδ2 (Vδ2) T cells have been proposed as cell carriers for off-the-shelf CAR therapies. Here the authors describe CD16 as a biomarker for the selection of Vδ2 T cells with high...

γδ T cells, constituting approximately 1% to 5% of total human peripheral T cells, are a crucial subset of T cells. Of these, approximately 80% to 85% express the TCR Vδ2 chain. Because the TCR Vδ2 chain is almost exclusively paired with the TCR Vγ9 chain, they are also known as Vγ9Vδ2 T cells[1]. Prior clinical trials employing Vδ2 T cells for adoptive transfer therapy, whether in autologous or allogeneic contexts, produced varied results. While certain cancer patients exhibited favorable therapeutic outcomes following this treatment, others showed no response. The incongruent findings underscore the necessity of identifying biomarkers capable of predicting responses to immunotherapy involving Vδ2 T cells[2].

Home
About the Community
Behind the Paper
News and Opinion
From the Editors
On the road
Community Guidelines
More about the Community
Instagram

Our objective was to develop CAR-Vδ2 T cell-based immunotherapy. Preceding CAR engineering, a series of killing assays were conducted using Vδ2 T cells sourced from diverse peripheral blood mononuclear cell (PBMC) donors and employed against various cancer cell lines. Striking variations in the tumor-killing abilities of Vδ2 T cells among donors prompted further investigation. Flow cytometry analysis revealed that donors with better performance exhibited a higher prevalence of $CD16^{+}$ Vδ2 T cells, and their Vδ2 T cells demonstrated elevated CD16 expression, categorizing them into the $CD16^{Hi}$ group. CD16 (FcγRIII), widely acknowledged as an IgG receptor facilitating antibody-dependent cell-mediated cytotoxicity (ADCC), is important for antitumor activities of many therapeutic antibodies. While the majority of CD16 research has centered on NK cells, some studies have reported CD16 expression by Vδ2 T cells[3]. Notably, Vδ2 T cells from the majority of donors expressed low CD16, forming the $CD16^{Lo}$ group, distinguishing them from NK cells.

When co-cultured with the ovarian cancer cell line OVCAR3 in the presence of Zoledronate (ZOL), an FDA-approved small molecule compound known to activate Vδ2 T cells[4], $CD16^{Hi}$ Vδ2 T cells exhibited elevated secretion of cytotoxic molecules, including perforin and granzyme B, as well as increased IFN-γ levels compared to $CD16^{Lo}$ Vδ2 T cells. This robust effector function may elucidate the superior tumor-killing capacity of $CD16^{Hi}$ Vδ2 T cells over $CD16^{Lo}$ counterparts. Notably, the overexpression of transgenic CD16 on $CD16^{Lo}$ Vδ2 T cells by lentiviral vectors did not augment their cytotoxic potential. This observation implies that CD16 could serve as a valuable biomarker for identifying donors with highly potent Vδ2 T cells, rather than functioning as an active receptor directly contributing to tumor killing. Furthermore, the genetic introduction of CD16 to $CD16^{Lo}$ Vδ2 T cells might not reproduce the potent antitumor activity observed in Vδ2 T cells expanded from $CD16^{Hi}$ donors.

Bulk RNA-Sequencing analysis further supported our *in vitro* assay results. CD16 expression positively correlated with immune effector and activation signatures, including cytotoxicity, degranulation, and innate immunity functions. Furthermore, the $CD16^{Hi}$ group demonstrated a downregulation of RORC, a transcription factor associated with a Th17-like phenotype of γδ T cells. Studies have shown that Th17-like γδ T cells can contribute to the progression of cancer in various syngeneic models, and there is supporting evidence for their adverse effects in human malignancies[5].

Home
About the Community
Behind the Paper
News and Opinion
From the Editors
On the road
Community Guidelines
More about the Community
Instagram

Encouraged by these findings, we then engineered CD16^{Hi} Vδ2 T cells with mesothelin-targeted CAR (MCAR) and soluble IL-15 using lentiviral vectors. Mesothelin, a cell-surface glycoprotein with minimal expression in normal tissue, exhibits overexpression in numerous solid tumors, including ovarian cancer[6]. Overexpression of transgenic soluble IL-15 has been shown to enhance the persistence of immune cells *in vivo* by many groups[7,8]. *In vitro* analyses demonstrated the multi-faceted tumor-targeting capabilities of the engineered Vδ2 T cells, encompassing CAR-mediated recognition, ZOL-induced activation of TCRs, and ADCC facilitated by CD16 in the presence of anti-HER2 therapeutic antibody, trastuzumab. This capacity for multi-targeting is crucial, especially given that tumor cells frequently downregulate the expression of their antigens. CAR-engineered CD16^{Hi} Vδ2 T cells may present a promising strategy to counteract situations of tumor antigen escape.

Based on the data demonstrating antitumor efficacy, we proceeded to assess the therapeutic cells in two ovarian cancer xenograft mouse models. Our results revealed that CD16^{Hi} Vδ2 T cells, engineered with MCAR and IL-15, exhibited not only superior antitumor efficacy but also sustained persistence in various mouse tissues, including ovarian tumors. Furthermore, the persistence of engineered Vδ2 T cells did not correlate with xenoreactivity. Histological examination of H&E-stained tissue sections from experimental mice indicated no tissue damage, suggesting that these therapeutic cells are unlikely to induce graft-versus-host disease (GvHD) in patients in allogeneic settings.

In response to current challenges posed by conventional CAR-based αβ T cell therapies, which exhibit limited efficacy against solid tumors, active exploration is underway to employ CAR-engineered innate immune cells, such as Vδ2 T cells. Vδ2 T cells exhibit characteristics from both the adaptive and innate immune systems, offering potential advantages over αβ T cells. These advantages include the ability to target tumors through multiple mechanisms, modulate the tumor microenvironment[9], and recruit other immune cells[10]. Nevertheless, donor-to-donor variations exist, with Vδ2 T cells from certain donors exhibiting superior antitumor functions compared to others. Our investigations suggest that CD16 has the potential to serve as a biomarker for the identification and selection of Vδ2 T cell donors.

We acknowledge that several questions could be addressed in future studies. $CD16^+$ and $CD16^-$ Vδ2 T cells could be isolated from $CD16^{Hi}$ Vδ2 T cell donors to facilitate a more comprehensive comparison of their gene expressions, phenotype, and functionality. It would be intriguing to further explore why some donors exhibit a higher frequency of $CD16^+$ Vδ2 T cells and why their CD16 expression is elevated. Additionally, conducting further analysis of $CD16^{Hi}$ Vδ2 T cells using single-cell RNA sequencing and epigenomic sequencing may unveil their etiology and contribute to the development of more refined $CD16^{Hi}$ Vδ2 T cell products.

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