

Improving the Anti-Cancer Effect Of T-Cells by T-Cell Receptor Engineering

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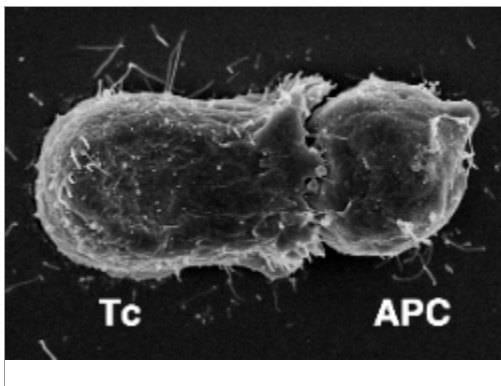
There are two populations of T-cells that are characterized by the type of T-cell receptor (TCR) that is expressed: $\alpha\beta$ and $\gamma\delta$. The majority of T-cells express $\alpha\beta$ TCRs while only 2% of total T-cells have TCRs made of $\gamma\delta$ subunits. Furthermore, a specific type of $\gamma\delta$ T-cell that expresses $\gamma9\delta2$ TCRs, a class of innate immune cells, can be highly anti-tumorigenic in mouse cancer models. However, adoptive transfer of $\gamma9\delta2$ cells in the clinic has provided less tumor control. The Strong (Basic Sciences Division) and Kuball (UMC Utrecht, The Netherlands) labs investigated two important questions: what is the molecular defect curtailing the anti-tumor effectiveness of $\gamma9\delta2$ T-cell therapy and can this weakness be ameliorated to increase their therapeutic potential?

As a starting point, $\gamma9\delta2$ T-cells from a healthy donor were cloned and their reactivity against a panel of tumor cells was assessed by an IFN γ ELISA assay. Individual clones of $\gamma9\delta2$ T-cells displayed differential recognition of and activation by specific tumor cells. They hypothesized that the strength of the anti-tumor response correlated with differences in $\gamma9\delta2$ TCR amino-acid sequence. To test this, the $\gamma\delta$ chains of a T-cell clone with strong functional avidity was sequenced and compared to a T-cell clone that displayed a weak response in the ELISA assay. Interestingly, only the CDR3 domain of the receptors contained amino acid changes. To determine if the amino acid changes influenced TCR function, the mutant receptors that displayed increased functionality and wild-type control receptors were transduced into peripheral blood $\alpha\beta$ T-cells and T-cell activity against tumor cells was determined. Indeed, $\alpha\beta$ T-cells expressing the stronger $\gamma9\delta2$ TCR had a greater ability to lyse tumor cells and secrete IFN γ compared to wild-type $\gamma9\delta2$ TCR-expressing $\alpha\beta$ T-cells. Thus, the CDR3 domain of the $\gamma9\delta2$ TCR is a critical determinant of TCR functionality.

Mutational analysis of CDR3 domain residues in both the $\gamma9$ and $\delta2$ chains of wild-type receptors revealed that too short (no amino acid addition) and very long (12 amino acid) alanine stretches in a particular CDR3 region negatively affect receptor function. To determine if an optimal CDR3 length exists, they searched the ImMunoGeneTics database for stretches of amino acids in the CDR3 regions of the $\gamma9\delta2$ TCRs. The majority of listed $\gamma9\delta2$ TCRs contained a conservative number of residues (5-7 amino acids) in the CDR3 domain, indicating that those TCRs have a functional advantage and are under positive selection. In addition, single point mutations in both chains of the

$\gamma\delta 2$ TCRs were found to influence TCR functional avidity. Lastly, CTE (combinatorial- $\gamma\delta$ TCR-chain-exchange) was used to engineer $\gamma\delta 2$ TCRs that were predicted to have increased receptor function based on the aforementioned results. Astoundingly, $\alpha\beta$ T-cells expressing the CTE-engineered $\gamma\delta 2$ TCRs were highly reactive against a broad range of tumors and did not affect normal tissue in the IFN γ ELISA. When tested in a humanized mouse model, the CTE-engineered $\gamma\delta 2$ TCRs decreased tumor outgrowth and increased the overall survival of the mice. Taken together, these data indicate that CTE-engineered TCRs are a promising candidate for clinical application and may prove useful for future anti-cancer therapy.

[Gründer C, van Dorp S, Hol S, Drent E, Straetemans T, Heijhuurs S, Scholten K, Scheper W, Sebestyen Z, Martens A, Strong R, Kuball J.](#) 2012. $\gamma 9$ and $\delta 2$ CDR3 domains regulate functional avidity of T-cells harboring $\gamma\delta 2$ T-cell receptors. *Blood*. Epub ahead of print, doi: 10.1182/blood-2012-05-432427.



Obtained from Institut Pasteur website.

A scanning electron micrograph of a T lymphocyte (Tc) recognizing and attaching to an antigen presenting cell (APC).