Improvement of CAR T-cell performance by simultaneous downregulation of multiple co-inhibitory receptors

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Background

- Co-inhibitory receptors, such as PD-1 and LAG-3, have a crucial role in regulating T-cell activity, as their expression on the cell surface upon chronic T-cell activation is associated with T-cell exhaustion.
- Immunocheckpoints directed against co-inhibitory receptors exhibited unprecedented efficacy in several cancer indications. However, many patients do not respond to these therapies and some tumor types remain largely refractory.
- A key determinant of CAR T-cell failure against solid tumors is T-cell exhaustion induced by checkpoint inhibitors on cancer cells.
- The coordinated targeting of different co-inhibitory receptors may help overcoming these hurdles.

Here we show that the simultaneous downregulation of co-inhibitory receptors PD-1, LAG-3, TIM-3 and death receptor CD95 improves the functionality of CAR T-cells.

Methods

A microRNA (miRNA)-based short hairpin RNA (shRNA) platform was developed, to allow for the tunable modulation of multiple target genes simultaneously. The platform was equipped with shRNA-derived guide sequences (shGuides) targeting PD-1, LAG-3, TIM-3 and CD95, and combined with a CD19 CAR. The engineered CAR T-cells were challenged with target cancer cells expressing the receptors’ ligands. The impact of the simultaneous downregulation of the four receptors was assessed in vitro by monitoring T-cell activation (via cytokine secretion), killing activity, and persistence (via repeated challenges with the target cells).

For further technical information regarding the miRNA-based shRNA platform, please see our recent publication in Molecular Therapy – Nucleic Acids.

Check also poster #298, where we use our platform to generate allogeneic CAR T-cells.

Results

1. Thanks to our shGuide screening platform, we could select efficient shGuides for each of the four targets.

In the case of the co-inhibitory receptors PD-1, LAG-3 and TIM-3, our shGuides could effectively silence the target gene expression upon the receptors’ upregulation following co-culture with cancer cells expressing the receptors’ ligands.

2. We screened a panel of 12 cancer cell lines for the expression of PD-1, LAG-3, TIM-3 and CD95 ligands, with the aim to find a cell line expressing ligands for all four receptors, that could therefore be used as a suitable model to assess the impact of our miRNA-based shRNA-4-plex on CAR T-cell functionality.

The ovarian adenocarcinoma cell line SK-OV-3 emerged as the best target cell candidate, thanks to its ligand expression profile.

3. The simultaneous downregulation of PD-1, LAG-3, TIM-3 and CD95 enhanced cytokine secretion compared to CAR T-cells not engineered with the miRNA-based shRNA platform, when challenged with SK-OV-3 cancer cells.

Likewise, the knock-down of the four surface markers in the CAR T-cells enhanced their killing activity against SK-OV-3 cancer cells.

4. CAR T-cell activity was scored following co-culture for 24h with SK-OV-3 cancer cells overexpressing CD19, by assessing cytokine secretion (IFNγ) by ELISA (left) and by monitoring killing activity via intracellular staining (right). Bars represent means ± SD of three biological replicates.

Affiliations & disclosures

Any questions? Please contact us at contact@celyad.com

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