Overcoming target-driven fratricide for CAR-T cell therapy

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**Background**

Chimeric antigen receptor (CAR) provides an approach to specifically target any tumor cell. The recent licensing of CAR T cell therapy for B cell acute lymphoblastic leukemia and diffuse large B cell lymphoma provides a strong clinical validation of the approach and an impetus to develop CAR T cell therapy beyond B cell malignancies. Success of the approach is largely dependent on the profile of the target antigen itself, whose broad tumor-associated antigens are not specific to the tumor. In certain circumstances, the target antigen may be constitutively or transiently expressed on a T cell, meaning that the CAR T cell may undergo self-fighting or fratricide.

A CAR is a fusion of the murine MUC1 protein with CD3ε or CD28(CD3ε- or CD28-based T cells with broad specificity for MHCII antigens, ensuring effective and self-targeting of a large variety of tumors (Figure 1). However, T cells transiently express three NKG2D ligands during activation (Figure 2) and consequently CD8+ CAR T cells undergo fratricide (Figure 3), thereby hampering the ability to exploit MUC1 as a therapy when high doses of cells are required. To address this we optimized the manufacturing conditions to which fratricide was to enable production of large number of functional cells to be used in clinical approaches.

**Figures & Tables**

**Figure 1: CYAD-01 CAR T construct**

**Figure 2: PI3K inhibition downregulates NKG2D expression, enhances CAR T cell viability, and reduces fratricide at 37°C due to high number of cells**

**Figure 3: NKG2D ligands are upregulated during T cell activation**

**Figure 4: PI3K inhibition downregulates NKG2D expression and modifies the T cell memory phenotype**

**Figure 5: Anti-NKG2D antibodies decrease the fratricide process in CAR T cell manufacturing.**

**Figure 6: Anti-NKG2D antibodies decrease the fratricide process in CAR T cell manufacturing.**

**Figure 7: Optimization of the process for expansion activity of PI3K inhibition and blocking antibody to CYAD-01 cell manufacturing**

**Results**

**Solution 1: PI3K inhibitor LY294002**

Given the plethora of NKG2D ligands that could be expressed on T cells, their elimination by gene-editing to inhibit fratricide in CAR-T cell manufacturing is challenging. The initial approach we employed was the inhibition of the PI3K-AKT axis using LY294002 (PI3K) into the production process. PI3K reduced NKG2D expression at the cell surface, partially inhibiting fratricide. This was more pronounced upon the freezing/thawing process (Figure 6). Furthermore, the PI3K silenced T-phenotype to central memory cells and reduced the production of high levels of cytokines (Figure 8).

**Solution 2: Blocking antibody (anti-CXCL10, clone 15102)**

While PI3K inhibition partially inhibited fratricide, impacted also cell growth, preventing the generation of the number of cells required for high dose levels (Figure 2). A target-specific approach involving depletion of the NK9.5 CAR T cell subset was employed and elicited a further improvement in the CYAD-T cell population blocking fratricide during the manufacturing process in a dose-dependent manner. (Figure 6). However, the CYAD-T cells produced with this process exhibited a minor delay in cytokine release, with reduced potency against cancer cell lines, due to identity of the CD4/CD8 ratio and the CD4+ cell ratio to the face of CD4+ T cells. (Figure 7).

Reduction of the culture protocol adjusting the incubation time of the blocking antibody and the length of culture showed that adding the blocking antibody at day 2 instead of directly after transduction day 1 was the reason to obtain CYAD-T cells with identical cytokine kinetics and IFNγ production ability, and comparable CYAD-T cells with cells produced using the PISK, while maintaining the optimal elicited expression (Figure 8).

**Conclusions & Perspectives**

* In the CAR T space, the choice of the target is crucial, ideally with a high expression at the surface of the tumor cells and no presence in healthy bodies. The multi-target specificity of the CYAD-01-based CAR (CYAD-01) ensures effective and self-targeting of a large variety of tumors. However, in the case of MUC1, like other target antigens permanently or transiently expressed on T cells, this leads to T cell fratricide, causing reduced cell yield. Gene editing approaches provide clinically relevant methods to prevent the expression of these antigens, thus inactivating fratricide and enabling CAR T cell expansion.

* While the target specificity of the CYAD-01-based CAR and the polyclonality of some of its receptors render gene editing particularly challenging.

* To tackle fratricide during CYAD-01 cell manufacturing, Celyad lately exploits PI3K inhibition, which turned out to partially respond to the need, but with limitations at high dose levels due to an opposing effect to cell proliferation. The use of a blocking antibody under a defined culture protocol was selected as a solution to achieve the right expansion to produce the high dose levels while maintaining a cytotoxic profile to the product manufactured using PI3K inhibition.

* This new formulation has been shown in clinical trials to determine the maximum tolerated dose in several indications.