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#119

Overcoming target-driven fratricide for CAR-T cell therapy

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BACKGROUND

Chimeric antigen receptor (CAR) provides an approach to putatively target any tumor cell. The recent licensing of CAR T cell therapy for B cell acute lymphoblastic leukemia and diffused 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. The success of the approach is largely dependent on the profile of the target antigen itself, where most known 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-killing or fratricide.

A CAR consisting of a fusion of the NKG2D protein with CD3zeta (CYAD-01) endows T cells with broad specificity for NKG2D ligands, ensuring effective and safe targeting of a large variety of tumors (**Figure 1**). However, T cells transiently express these ligands during activation (**Figure 2**) and consequently CYAD-01 CAR T cells undergo fratricide (**Figure 3**), thereby hampering the ability to exploit NKG2D as a therapy when high doses of cells are required. In this work we optimized the manufacturing conditions to inhibit fratricide and to enable production of large number of functional cells to be used in clinical approaches.

FIGURES & TABLES

FIGURE 1: CYAD-01 CAR T construct

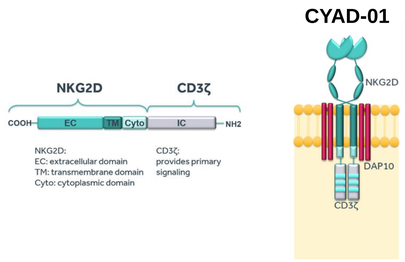


FIGURE 2: NKG2D ligands are upregulated during T cell activation

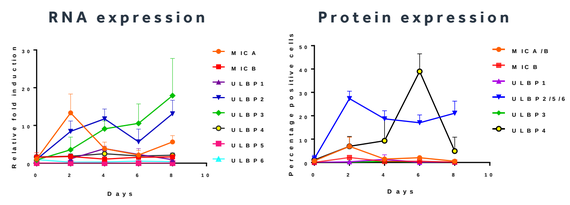


FIGURE 3: CYAD-01 cells undergo fratricide during manufacturing, which affects the CD4/CD8 ratio and inhibits expansion

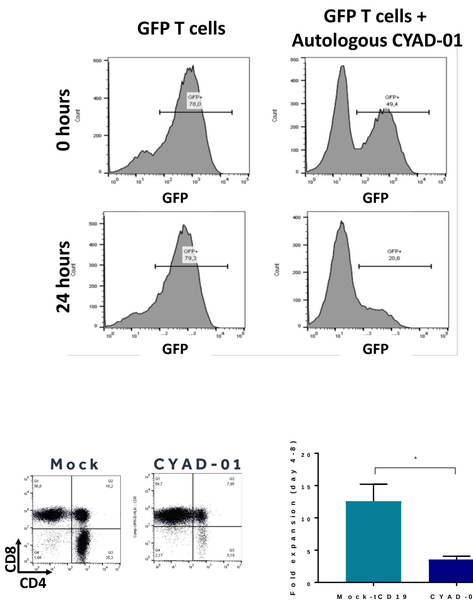
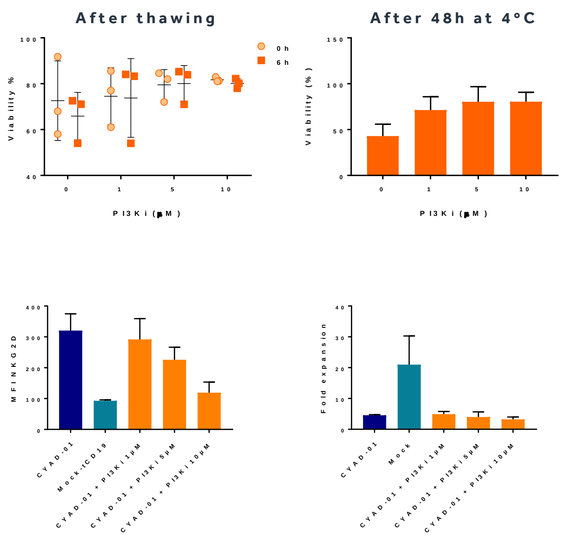


FIGURE 4: PI3K inhibition downregulates NKG2D expression, thus increasing viability of CYAD-01 cells mainly after cryopreservation and upon maintenance at 4°C, but fails to produce high number of cells



RELEVANT LITERATURE:

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FIGURE 5:PI3K-inhibition boosts cytokine production and modifies the T cell memory phenotype

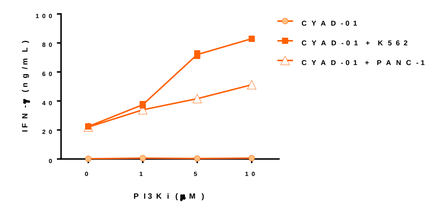


FIGURE 6: Antibody-mediated blocking of NKG2D prevents CYAD-01 fratricide and allows production of high doses of potent CYAD-01 cells

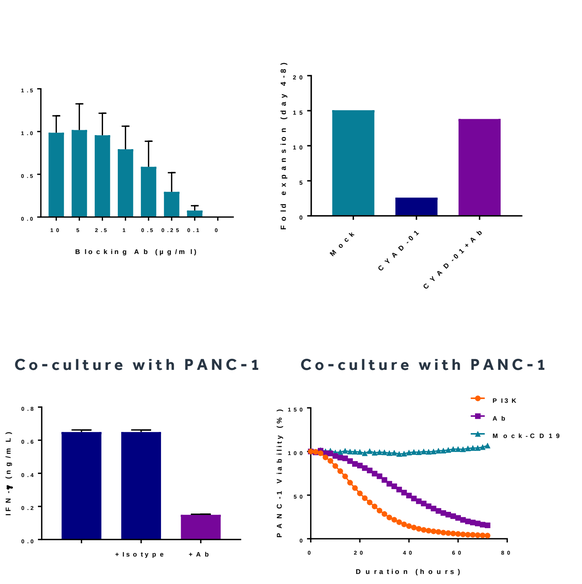
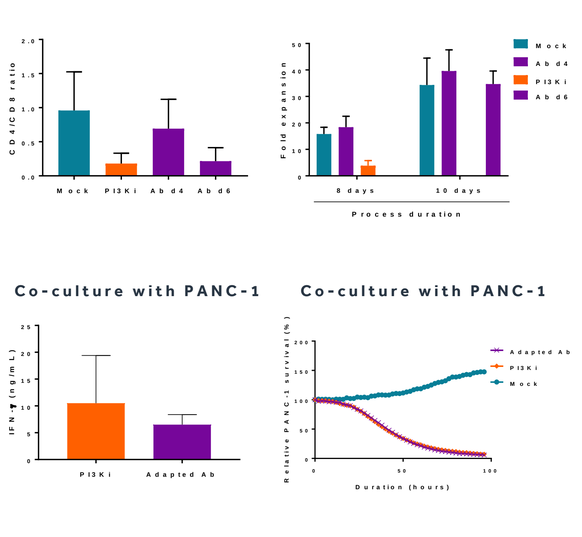


FIGURE 7: Optimization of the process for equivalent activity of PI3K inhibition and blocking antibody in CYAD-01 cell manufacturing



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RESULTS

SOLUTION 1: PI3K inhibitor LY294002

Given the plethora and the polymorphism of NKG2D ligands that could be expressed on T cells, their elimination by gene editing to inhibit fratricide in CYAD-01 cells is challenging. The initial approach we employed was the inclusion of the Phosphoinositol-3-Kinase inhibitor LY294002 (PI3Ki) into the production process. PI3Ki reduced NKG2D expression at the cell surface, partially inhibiting fratricide. This was more pronounced upon the freezing/thawing process (**Figure 4**). Furthermore, the PI3Ki skewed T cell phenotype to central memory cells and induced the production of high levels of cytokines (**Figure 5**).

SOLUTION 2: Blocking antibody (anti-CD314, clone 1D11)

While PI3K inhibition partially inhibited fratricide, impacted also cell growth, preventing the generation of the number of cells required for high dose levels (**Figure 4**). A target-specific approach involving blockade of the NKR-2 CAR itself was employed and elicited a further improvement in the CYAD-01 cell yield by blocking fratricide during the manufacturing process in a dose-dependent manner (**Figure 6**). However, the CYAD-01 cells produced with this process exhibited a minor delay in cytolytic kinetics, with reduced potency against cancer cell lines, due to skewing of the CD4/CD8 cell ratio to the favor of CD4 T cells (**Figure 7**). Modifications 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 6 instead of directly after transduction (day 4) was the means to obtain CYAD-01 cells with identical cytolytic kinetics and IFNγ production ability, and comparable CD4/CD8 ratios with cells produced using the PI3Ki, while maintaining the optimal fold expansion (**Figure 7**).

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 tissues. The multi-target specificity of the NKG2D-based CAR (CYAD-01) ensures effective and safe targeting of a large variety of tumors. However, in the case of NKG2D, like with 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 those antigens, thus inhibiting fratricide and enabling CAR T cell expansion. However, the multi-target specificity of the NKG2D-based CAR and the polymorphism of some of its receptors render gene editing particularly challenging.
- To tackle fratricide during CYAD-01 cell manufacturing, Celyad initially used a PI3K inhibitor, 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 similar profile to the product manufactured using the PI3K inhibitor.
- This new formulation is now used in clinical trials to determine the maximum tolerated dose in several indications.