Chimeric antigen receptor (CAR) T-cell therapy has now become a realistic first-line treatment option for patients with some B-cell malignancies. Still, several limitations remain, including those associated with the time-consuming and highly personalized manufacturing of autologous CAR T-cells. Off-the-shelf allogeneic CAR T-cells derived from healthy donor cells have the potential to overcome these challenges. However, adoptive transfer of allogeneic T-cells carries the risk of graft-versus-host disease (GVHD).

In order to mitigate this risk, further engineering of the CAR T-cells is needed. We present here shRNA interference to knockdown the expression of the CD3ζ component of the T-cell receptor (TCR) at the mRNA level in CAR T-cells targeting the B-cell maturation antigen (BCMA) to treat multiple myeloma (MM).

The shRNA against CD3ζ significantly reduced the CD3ζ mRNA expression in both Jurkat and primary T-cells (Figure 1B and C), leading to functional inhibition of the TCR complex, when stimulated with CD3 targeting antibodies (Figure 1D).

When expressed together with a BCMA-specific CAR into donor T-cells to generate the CYAD-211 clinical product (Figure 2A), the insertion of the anti-CD3ζ shRNA efficiently knocks down cell surface TCR expression to undetectable levels (Figure 2B).

The lack of weight loss (Figure 3A), and survival of mice (Figure 3B), following the infusion of CYAD-211 in sublethally irradiated NSG mice, as compared to mice infused with control Mock T-cells, demonstrated the lack of alloreactivity of CYAD-211 in vivo (no evidence of GVHD).

Anti-tumor efficacy of CYAD-211 was confirmed in vivo in xenograft multiple myeloma models, where long-term survival was observed (Figure 3C) and reduced tumor burden (Figure 3D), as compared to mice infused with control Mock T-cells.

CYAD-211 was evaluated in an open-labeled multi-center Phase 1 trial (NCT04613557) in adult MM patients with refractory or relapsed disease to at least 2 prior MM treatment regimens. Twelve patients received a single infusion of CYAD-211 at three different dose-levels (3x10^7, 1x10^7 and 3x10^6 cells/infusion) administered after a non-myeloablative preconditioning chemotherapy (300 mg/m² cyclophosphamide, 30 mg/m² fludarabine daily for 3 days) (Table 1).

Overall, CYAD-211 demonstrated an acceptable safety profile with no dose limiting toxicity (DLT), GVHD or CAR-T-cell-related encephalopathy syndrome (CRES) (Table 2), demonstrating the safety of the shRNA-based approach to prevent the GVHD risk. Three patients achieved partial response (PR), one in each dose-level, while eight patients had stable disease (SD) (Figure 4A).

All patients had detectable CYAD-211 cells in the peripheral blood. However, the engraftment was short lasting (Figure 4B). Clearance of CYAD-211 cells was correlated with recovery of endogenous white blood cell (WBC) population over time (Figure 4C), suggesting that the CAR T-cells disappearance was due to Host versus Graft (HVg) reaction.

CONCLUSIONS

• The co-expression of a shRNA against CD3ζ together with a CAR can be used to design allogeneic CAR T-cells without compromising their anti-tumor activity in vitro and in vivo and demonstrating a lack of alloreactivity in preclinical models.

• Data from the IMMUNICY-1 trial provide proof of concept that single shRNA-mediated knockdown can fully functional allogeneic CAR T-cells in humans without any signs of GVHD, while maintaining a good safety profile and efficacy.

• This supports the use of this non-gene editing technology to engineer 'off-the-shelf' allogeneic CAR T-cells, but also to engineer CAR T-cells with improved characteristics (e.g. persistence, resistance to immunosuppression...).

• We are currently validating the technology to downregulate multiple-genes of interest simultaneously thereby providing a platform approach that could support the future of cell therapy [1] [see also posters #237 and #238].

TABLES & FIGURES

Figure 1: The shRNA against CD3ζ impairs the TCR activity

Figure 2: CYAD-211 clinical product

Figure 3: CYAD-211 demonstrates high anti-tumor efficacy without evidence of GVHD in preclinical models in vivo

Figure 4: CYAD-211 induced signs of clinical activity in patients with multiple myeloma but showed limited persistence

Table 1: Demographics and clinical characteristics of patients recruited in the IMMUNICY-1 trial

Table 2: CYAD-211 showed no evidence of GVHD in patients with multiple myeloma

REFERENCES

1. Rossi et al., 2023. Mol Ther – Nucleic Acids 34:102038

AFFILIATIONS, DISCLOSURES & ACKNOWLEDGMENTS

All authors are employed by Celyad Oncology SA. We thank the patients who participated in the study, their families, friends, and caregivers, and the study staffs and health-care providers at all the clinical sites. We acknowledge the work of all other principal investigators i.e. Sebastien Anguille, A Samer Al-Homsy, Dries Deeren, Taiga Nishihori and Nathalie Meuleman, who contributed to recruit and treat patients, and we thank them for their commitment and support during the study conduct. This poster is published for information only.