Proof-of-concept of a non-gene editing technology using shRNA down-regulation to engineer allogeneic CAR T-cells

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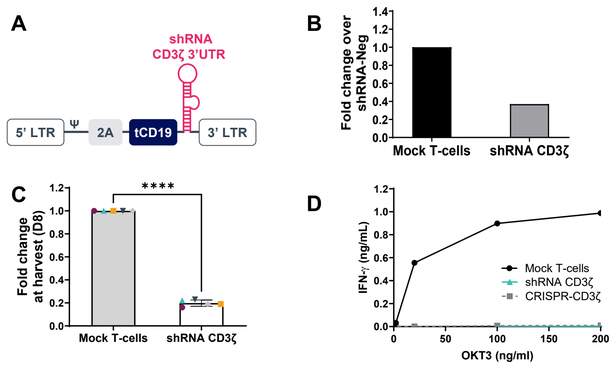
BACKGROUND

- 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 short hairpin RNA (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).

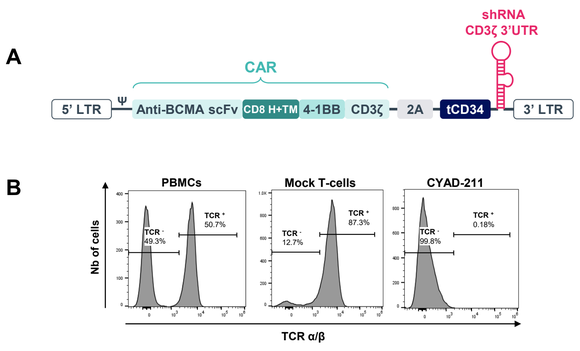
TABLES & FIGURES

Figure 1: The shRNA against CD3ζ impairs the TCR activity



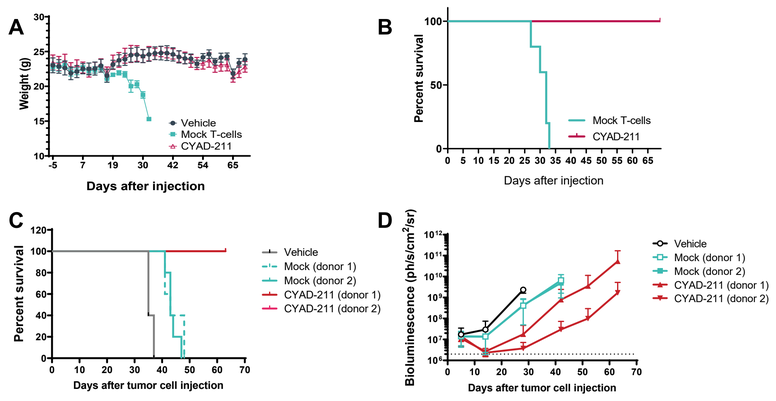
A. Schematic representation of the shRNA CD3ζ genetic construct. **B.** Impact on mRNA levels of CD3ζ in Jurkat cells after transduction, compared to untransduced control cells, evaluated by qPCR at harvest. **C.** Impact of the shRNA transduction on CD3ζ mRNA levels in primary T-cells (5 donors). Data for each of the T-cell arms are presented as mean +/- SD. **** $p < 0.0001$. **D.** IFN-γ secretion in the supernatant of primary T-cells from a representative healthy donor (out of 5) transduced with the shRNA CD3ζ vector, or control vector (Mock T-cells) or nucleofected with CRISPR against CD3ζ after 24-hour incubation with the anti-CD3 antibody (OKT3).

Figure 2: CYAD-211 clinical product



A. Schematic representation of CYAD-211 genetic construct. **B.** Representative flow cytometry histogram plots of TCR expression of peripheral blood mononuclear cells (PBMCs), control Mock T-cells and CYAD-211 product, illustrating the lack of TCR expression on CYAD-211 cells. H, Hinge; LTR, long-terminal repeat; scFv, single-chain variable fragment; tCD34, truncated non-functional form of CD34 membrane protein; TM, transmembrane; 2A, 2A furin-mediated cleavage site and self-cleaving peptide; UTR, untranslated region; ψ, retroviral Psi packaging element.

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



A and B. Weight kinetics (A) and survival curve (B) of immunodeficient (NSG) mice (n=5 per group) injected i.v. with vehicle, or $2x10^7$ CYAD-211 or control T-cells, 1 day following sublethal (1.44 Gy) total body irradiation. **C and D.** Survival curve (C) and kinetics of bioluminescence (D) of tumor-bearing immunodeficient (NSG) mice (n=5 per group) injected intravenously (i.v.) with vehicle, or 10^7 CYAD-211 or control T-cells, 6 days following i.v. injection of $5x10^6$ KMS-11 multiple myeloma (MM) cells.

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

Characteristics	DL1 (N=3)	DL2 (N=3)	DL3 (N=6) *	Total (N=12)
Age, median (range), (years)	72 (57-78)	63 (49-70)	69 (55-82)	68.5 (49-82)
Gender, %, (Male/Female)	67/33	33/67	50/50	50/50
ECOG score at screening, %, 0/1	67/33	67/33	83/17	75/25
R-ISS Stage at screening, %, 1/2/3	0/67/33	0/67/33	17/50/33	8/58/33
mSMART risk: High [1] (%)	50	67	67	64
Extramedullary disease (%)	0	33	33	25
Time between diagnosis and CYAD-211 infusion, median (range), (years) [2]	6.8 (6.4-7.5)	7 (3.1-7.3)	6 (2.6-22.2)	6.9 (2.6-22.2)
Prior lines of therapy, median (range), (number)	4 (2-4)	4 (3-4)	4.5 (2-6)	4 (2-6)
Prior autologous SCT (%)	67	100	83	83
At least triple-exposed (%)	67	100	83	83

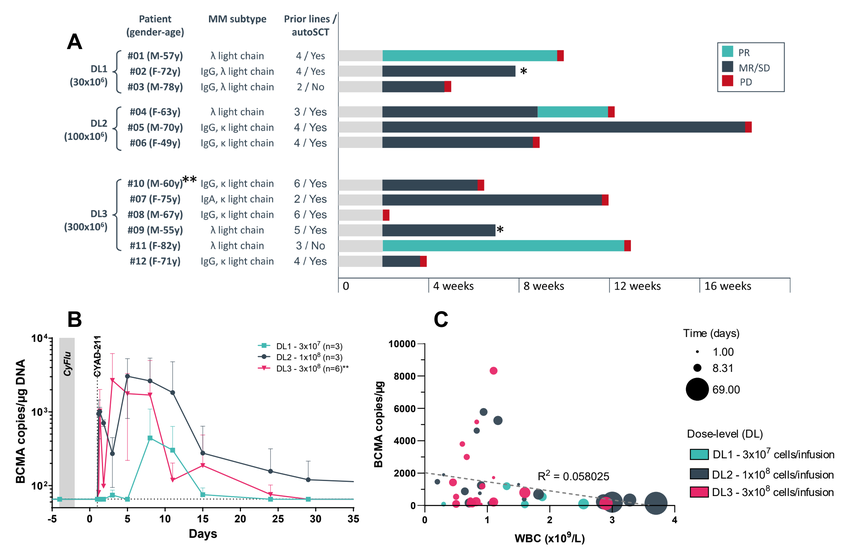
[1] High risk genetics abnormalities according to mSMART 3.0 are t(4;14), t(14;16), t(14;20), del 17p, p53 mutation and gain 1q; [2] Incomplete dates of diagnosis were replaced by the first day of the month of diagnosis. * One patient was enrolled in Cohort 3 but due to a human error while preparing the CYAD-211 infusion, the patient received a third of a dose-level 3, corresponding to a dose-level 2. ECOG: Eastern Cooperative Oncology Group; ISS: International Staging System; SCT: stem cell transplantation.

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

	Nb of patients (N = 12) presenting AE of interest (highest grade)					
AE of interest	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	All Grades
AE related to CYAD-211	9 (75%)	6 (50%)	4 (33%)	3 (25%)	1 (8%)*	10 (83%)
SAE related to CYAD-211	1 (8%)	-	1 (8%)	-	1 (8%)*	2 (17%)
Cytokine release syndrome (CRS) [1]	1 (8%)	-	-	-	-	1 (8%)
CAR-T cell-related encephalopathy syndrome [1]	-	-	-	-	-	-
Graft-versus-host disease (GvHD)	-	-	-	-	-	-
Infection [2]	2 (17%)	1 (8%)	1 (8%)	-	1 (8%)*	5 (42%)
Infusion reaction to CYAD-211	-	-	-	-	-	-

[1] As per CAR-T-cell-therapy-associated TOXicity (CARTOX) Working Group criteria from Neelapu (2018) Nat Rev Clin Oncol. 15(1):47-62. [2] Including bacterial, viral and fungal infections. AE: Adverse Event; SAE: serous adverse event. * One patient had a reported grade 5 Herpes Simplex Virus [HSV] encephalitis considered as possibly related to CYAD-211 by the Investigator but the event of death happened 10 months after infusion and the batch infused to the patient was negative for HSV.

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



A. Response and duration of response until progression in IMMUNICY-1 trial **B.** CYAD-211 cell kinetics as determined by digital droplet polymerase chain reaction. **C.** Bubble graph representation of the interactions between CYAD-211 cell kinetics, absolute white blood cells (WBC) count and time post infusion. The dotted line represents the multiple variable linear regression curve fitting data approximation.
* Patient discontinuation prior to disease progression; ** One patient received a third of a dose-level 3, corresponding to a dose-level 2.

TABLES & FIGURES

- 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 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 ($3x10^7$, $1x10^8$ and $3x10^8$ 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 generate 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 #297 and #298**].

AFFILIATIONS, DISCLOSURES & AKNOWLEDGMENTS

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 study sites. We acknowledge the work of all other principal investigators i.e. Sebastien Anguille, A Samer Al-Homsi, 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.

Any question? Please contact us at contactus@celyad.com

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