Clinical Development of a Non-Gene-Edited Allogeneic BCMA-Targeting CAR T-cell Product in Relapsed or Refractory Multiple Myeloma

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BACKGROUND

- Multiple myeloma (MM) remains largely incurable. Despite demonstrated efficacy of current therapies, most patients eventually relapse due to persistence of therapy-resistant plasma cell clones in the bone marrow.
- Autologous chimeric antigen receptor (CAR) T cell therapy directed against the B-cell maturation antigen (BCMA), a protein that is expressed on MM cancer cells, has shown impressive objective response rates in patients with advanced MM.
- Clinical grade manufacturing of autologous CAR T cells has limitations including vein-to-vein delivery time delay and potential sub-optimal immunological capability of T-cells isolated from patients with advanced disease. Allogeneic CAR T cell products, whereby cells from healthy third-party donors are used to generate an "off-the-shelf" product, have the potential to overcome some of these issues.
- **CYAD-211** is an **"off-the-shelf" non-gene edited allogeneic anti-BCMA CAR** T-cell product which uses an **All-in-One vector approach** (**Figure 1**) to co-express:
  - A CAR made by the in-line fusion of a BCMA-specific single-chain variable fragment (scFv) with the extracellular hinge, transmembrane domain of CD8α, and the intracellular domains of 4-1-BB and CD3ζ
  - A single optimized short hairpin RNA (shRNA) to down-regulate the expression of the T-cell receptor (TCR) CD3ζ subunit, thereby impairing the TCR expression on the surface of the donor T-cells, preventing their alloreactivity with the normal host tissue cells and potential risk of life-threatening graft-versus-host disease (GvHD)
  - A truncated human CD34 membrane protein as an identification and selection marker
- CYAD-211 is Celyad Oncology's first allogeneic CAR T candidate using shRNA through an All-in-One vector approach.

PRECLINICAL RESULTS

- The shRNA targeting CD3ζ incorporated in the CYAD-211 vector efficiently knocks down cell surface TCR expression to undetectable levels in the final CAR T cell product (**Figure 3A**) concurrent with inhibiting functional T cell responses to TCR activating stimuli (**Figure 3B**).
- Upon co-culture with the BCMA-expressing multiple myeloma cell lines RPMI-8226, OPM-2 and KMS-11, CYAD-211 cells produced high amounts of interferon-gamma (IFN-γ) and exhibited potent cytotoxic activity, in contrast to control cells produced from the same donors, bearing only the selectable marker or the selectable marker and the shRNA targeting CD3ζ without the BCMA-targeting CAR (**Figure 4**).
- The anti-tumor efficacy of CYAD-211 was further confirmed in vivo in xenograft MM models using the KMS-11 (**Figure 5A**) and RPMI-8226 (data not shown) cell lines. Immunodeficient (NSG) mice injected with vehicle or control T-cells succumbed to tumor with a median survival of 35 and 43 days, respectively, while mice injected with CYAD-211 cells remained alive until the end of the experiment, illustrating effective anti-tumor activity of CYAD-211 cells.
- No demonstrable evidence of GvHD when CYAD-211 was infused in sublethally irradiated NSG mice, the gold standard preclinical model of GvHD, confirming efficient inhibition of alloreactivity. While mice injected with CYAD-211 cells did not show any evidence of weight loss, the first sign of GvHD in this animal model, and survived until the end of the experiment (day 69), mice injected with control cells generated from the same donor manifested weight loss as of Day 23 and median survival of 32 days (**Figure 5B, 5C**). These results demonstrate that CYAD-211 cells effectively protect against induction of GvHD.

FIGURES

Figure 1: CYAD-211 vector

BCMA, B-cell maturation antigen; H, Hinge; LTR, long-terminal repeat; shRNA, short hairpin ribonucleic acid; 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 2: Treatment schedule in the IMMUNICY-1 study

Figure 3: CYAD-211 exhibit undetectable levels of TCR and do not respond to TCR activation signals

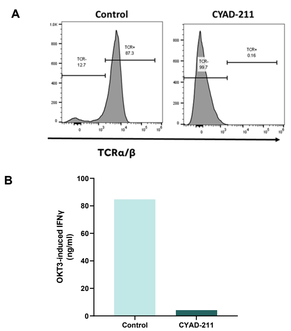
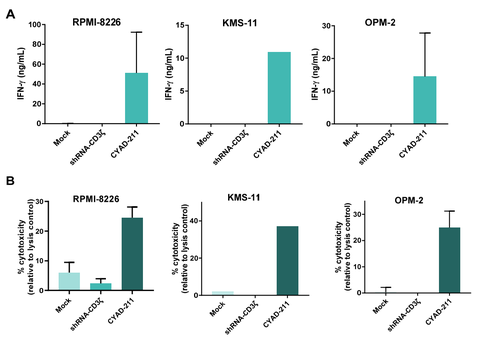


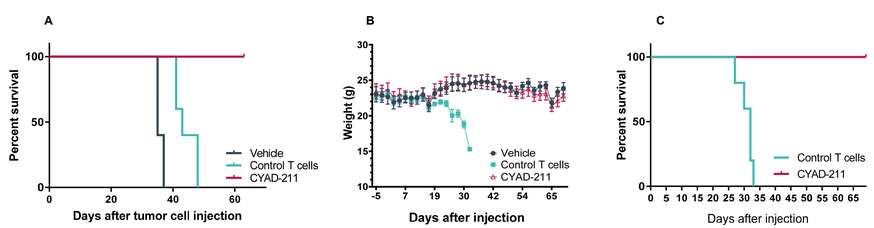
Figure 4: CYAD-211 exhibit robust *in vitro* anti-tumor activity



A. Representative flow cytometry histogram plots of TCR expression of control cells (left panel) and CYAD-211 CAR T cell product (right panel), illustrating the lack of TCR expression on CYAD-211 cells.
B. IFN-γ secretion in the supernatant of control and CYAD-211 cells generated from 3 independent healthy donors after a 24-hour incubation with 200ng/ml OKT3.

IFN-γ secretion (panel **A**) and cytotoxic activity (panel **B**) measured using ELISA and LDH assay respectively, in the supernatant of 24-hour 1:1 ratio co-cultures of T cells with the indicated multiple myeloma cell lines.
T cells were generated from 7 independent healthy donors upon transduction with a vector bearing only the reporter gene (Mock), the reporter gene and the shRNA targeting CD3ζ (shRNA-CD3ζ) or the CYAD-211 construct.

Figure 5: CYAD-211 demonstrates high anti-tumor activity without signs of alloreactivity in preclinical models *in vivo*



A. Survival curve 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 MM cells.
B, C. Weight kinetics (**B**) and survival curve (**C**) 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.

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4. AZ Delta, Roeselare, Belgium; 5. Institut Jules Bordet (ULB), Brussels, Belgium; 6. Celyad Oncology, Mont-Saint-Guibert, Belgium
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IMMUNICY-1 STUDY

- The first-in-human study evaluating CYAD-211, the open-label Phase I IMMUNICY-1 study (**NCT04613557**), has been initiated and first patient to be enrolled in November 2020.
- **Study design**:
  - Dose escalation segment with a 3+3 design evaluating three dose levels (DL) of CYAD-211: $3x10^7$, $1x10^8$ and $3x10^8$ to define the recommended dose; 6 patients will be enrolled in total at recommended dose
  - DLs of CYAD-211 were selected based on previous studies with autologous anti-BCMA CAR T cell products and studies with gene-edited allogenic CAR T-cell products
  - Potential higher DLs of CYAD-211 might be evaluated if the maximum tolerated dose is not reached
- **Treatment schedule (Figure 2)**:
  - Single infusion of the allogeneic CYAD-211 anti-BCMA CAR T cell product at Day 1 administered after a non-myeloablative preconditioning chemotherapy
  - Preconditioning chemotherapy consisting of 300 mg/m² cyclophosphamide and 30 mg/m² fludarabine daily for 3 days (CyFlu, at Days -5 to -3) aims to improve expansion of CYAD-211 cells and subsequently their persistence and clinical activity
- **Key inclusion criteria**:
  - MM with relapsed or refractory (r/r) disease to at least two prior MM treatment regimens which should include exposure to immunomodulatory drug (IMiD) and proteasome inhibitor (PI) either alone or in combination
  - At least 1 complete cycle of treatment for each prior treatment regimen
  - At least 1 response to a prior treatment regimen
  - Measurable disease as per the International Myeloma Working Group (IMWG) Response Criteria
- **Primary objective**
  - Determine the recommended dose (RecD)
- **Study endpoints**:
  - Primary endpoint of the dose escalation segment is the occurrence of dose-limiting toxicities (DLT) after the CYAD-211 infusion
  - Key secondary and exploratory endpoints include additional safety parameters, objective responses and duration of responses, and CYAD-211 cell kinetics in peripheral blood, bone marrow and extramedullary tumor sites/plasmacytoma

CONCLUSIONS

- CYAD-211 is the first non-gene edited allogeneic CAR T cell product based on shRNA technology, incorporating a BCMA-targeting scFv and a shRNA targeting the CD3ζ subunit of TCR complex.
- CYAD-211 demonstrated robust anti-tumor activity *in vitro* and *in vivo* concurrent with lack of alloreactivity in preclinical models.
- The preclinical data supported the initiation of the first-in-human study (IMMUNICY-1) with CYAD-211 in r/r MM patients.
- The IMMUNICY-1 clinical study seeks to provide proof of principle that single shRNA-mediated knockdown can generate fully functional allogeneic CAR T cells in humans without GvHD-inducing potential.
- We anticipate that subsequent generations of this technology will incorporate multiple shRNAs within a single vector system. Maintaining all the elements required for the therapy within a single vector (All-in-One vector) provides significant manufacturing advantage, necessitating single transduction and single selection step.

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