Poster 3844

Interim results from the phase I DEPLETHINK trial evaluating the infusion of a NKG2D CAR T-cell therapy post a non-myeloablative conditioning in relapsed or refractory acute myeloid leukemia and myelodysplastic syndrome patients

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BACKGROUND

- The rapid approval of two anti-CD19 **chimeric antigen receptor** (CAR) T-cell therapies and advanced development of anti-BCMA CAR T-cell therapy demonstrates the potential of the approach in B-cell malignancies. However, targets with a similar profile for CAR T-cell therapy in other diseases including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are lacking.
- **CYAD-01** is an **autologous** CAR T-cell therapy engineered with **a multi-complex, second-generation NKG2D CAR** comprising the full-length human natural killer group 2D (NKG2D) receptor fused to the intracellular domain of CD3ζ.
- The **NKG2D** receptor targets 8 ligands (MHC class I chain related proteins A [MICA] and B [MICB] and unique long 16 binding proteins [ULBP] 1–6 ligands) found at high frequency across a range of malignancies. Interestingly, non-malignant cells within the tumor microenvironment (myeloid-derived suppressor cells, regulatory T-cells and neo-endothelial cells) also express NKG2D ligands which led in preclinical models to the induction by CYAD-01 of a broader anti-tumor response beyond direct cancer cell killing.
- **CYAD-01** is being evaluated in relapsed/refractory (r/r) AML/MDS patients, with the objective to define whether the optimal CYAD-01 treatment is with prior preconditioning chemotherapy (DEPLETHINK study, here discussed) or without any pretreatment (see THINK study, **poster 3826**).

DEPLETHINK STUDY

- The open-label Phase I/II **DEPLETHINK Study** (NCT03466320) evaluates a single infusion of the autologous CYAD-01 administered after a non-myeloablative preconditioning chemotherapy in r/r AML or MDS patients.
- The preconditioning chemotherapy consists of 300 mg/m² cyclophosphamide and 30 mg/m² fludarabine daily for 3 days (CyFlu). This preconditioning chemotherapy should (i) favor the proliferation and expansion of CAR T-cells and (ii) increase the NKG2D ligand expression in tumor tissues targeted by CYAD-01.
- **Dose escalation** segment with a Fibonacci 3+3 design:
  - Three dose levels (DL) of CYAD-01: $1x10^8$, $3x10^8$ and $1x10^9$ cells per infusion administered as a single infusion after the preconditioning chemotherapy,
  - The first DL of CYAD-01 was selected at low dose of cells (~ $1.5x10^6$/kg) for safety precaution as first-time-in human infusion of an NKG2D CAR T-cell post chemotherapy,
  - The first DL of CYAD-01 was evaluated at two intervals between preconditioning and CYAD-01 infusion (last preconditioning treatment administered 7 days (T7) or 3 days (T3) before CYAD-01 infusion) to evaluate any potential modulation of the CYAD-01 cell engraftment,
  - Potential CYAD-01 consolidation cycle of 3 infusions every two weeks without prior preconditioning chemotherapy in the absence of progressive disease (PD) one month after the first CYAD-01 infusion.
- **Primary endpoint** of the dose escalation segment is the occurrence of dose-limiting toxicity (DLT) during the CYAD-01 treatment phase. Key secondary endpoints include additional safety parameters, CYAD-01 cell kinetics, objective responses and duration of responses.
- A potential **Phase II segment** is planned according to specific futility analysis at the end of the dose escalation segment.

TABLES & FIGURES

Table 1: Patient characteristics

Study snapshot: 19 Nov 2019	All patients N = 9	DL-1 $1x10^8$ N=6	DL2 $3x10^8$ N=3
Age (years): Mean (Range)	63.6 (50-73)	61.7 (50-73)	67.3 (58-73)
Gender: Male/Female	6/3	4/2	2/1
ECOG performance score at screening (Grade 0/1/2)	5/3/1	4/2/0	1/1/1
LVEF (%): Mean (Range)	57.7 (45-66)	56.5 (45-65)	60.0 (54-66)
Tumor type			
r/r Acute Myeloid Leukemia	7	4	3
r/r Myelodysplastic Syndrome	2	2	0
ELN 2017/R-IPSS Risk Stratification for AML/MDS			
Favorable (AML)/Intermediate (MDS)	0/0	0/0	0/0
Intermediate (ALM)/High-Risk (MDS)	3/0	1/0	2/0
Adverse (AML)/Very High-Risk (MDS)	4/2	3/2	1/0
Bone marrow blasts (%) mean (range)	26.0 (3.0-48.0)	28.0 (6.0-48.0)	22.0 (3.0-38.0)
Platelets (10^3/µL) mean (range)	64.2 (21.0-156.0)	80.2 (45.0-156.0)	32.3 (21.0-43.0)
ANC (10^3/µL) mean (range)	1.83 (0-8.7)	2.7 (0-8.7)	0.2 (0-0.38)

Figure 1: CYAD-01 kinetics in the peripheral blood (highest peak)

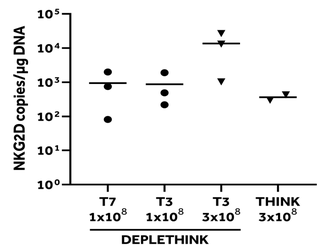


Table 2: Incidence of treatment-related adverse events (AEs) in patients infused with CYAD-01 produced with the current mAb manufacturing process

Study snapshot: 19 Nov 2019	DL-1 (T3 and T7 intervals)						DL-2 (T3 interval)					
	INDUCTION with preconditioning ($1x10^8$) N=6 (6 infusions)			CONSOLIDATION without preconditioning ($1x10^9$ or $3x10^9$) N=2 (4 infusions)			INDUCTION with preconditioning ($3x10^8$) N=3 (3 infusions)			CONSOLIDATION without preconditioning ($3x10^9$) N=1 (1 infusion)		
Adverse Event (AE) Preferred Term	**All Grades**	**Grade 3**	**Grade 4**	**All Grades**	**Grade 3**	**Grade 4**	**All Grades**	**Grade 3**	**Grade 4**	**All Grades**	**Grade 3**	**Grade 4**
Total of patients with at least 1 related AE (%)	3 (50.0%)	-	-	2 (100.0%)	1 (50.0%)[1]	1 (50.0%)[1]	1 (33.3%)	-	-	1 (100.0%)	1 (100.0%)	-
Cytokine release syndrome	3 (50.0%)	-	-	2 (100.0%)	-	1 (50.0%)[1]	1 (33.3%)	-	-	1 (100.0%)	1 (100.0%)	-
Encephalopathy	-	-	-	1 (50.0%)	1 (50.0%)[1]	-	-	-	-	-	-	-
Hyperglycaemia	-	-	-	1 (50.0%)	1 (50.0%)[1]	-	-	-	-	-	-	-
Abdominal Distension	-	-	-	1 (50.0%)	-	-	-	-	-	-	-	-
Agitation	-	-	-	1 (50.0%)	-	-	-	-	-	-	-	-
Chills	-	-	-	-	-	-	-	-	-	1 (100.0%)	-	-
Cough	-	-	-	-	-	-	-	-	-	1 (100.0%)	-	-
Diarrhoea	1 (16.6%)	-	-	-	-	-	1 (33.3%)	-	-	-	-	-
Disseminated intravascular coagulation	-	-	-	1 (50.0%)	-	-	-	-	-	-	-	-
Hyperphosphataemia	-	-	-	1 (50.0%)	-	-	-	-	-	-	-	-
Hypocalcaemia	-	-	-	1 (50.0%)	-	-	-	-	-	-	-	-
Non-cardiogenic pulmonary oedema	-	-	-	-	-	-	-	-	-	1 (100.0%)	-	-
Sepsis syndrome	-	-	-	-	-	-	-	-	-	1 (100.0%)	-	-

[1] Consolidation cycle at the $3x10^9$ cells/infusion

Figure 2: CYAD-01 CD8+ T-cells manufactured with the mAb or OptimAb process present an early phenotype (CD45RA/CD27 labeling)

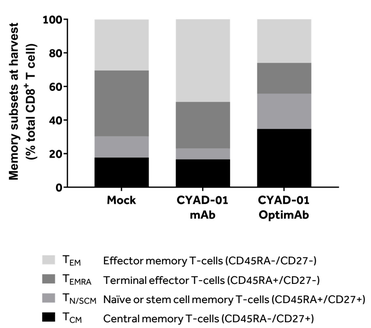


Figure 3: CYAD-01 CD8+ T-cells manufactured with the mAb or OptimAb process present an early phenotype (CD62L labeling)

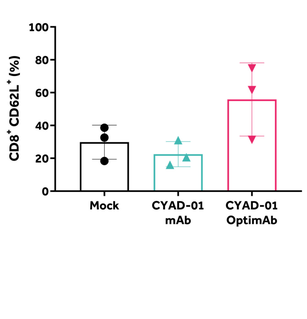
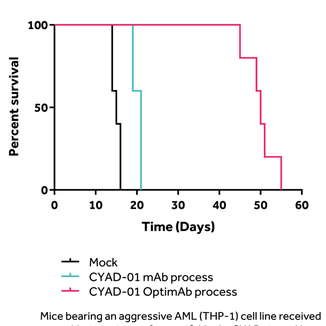


Figure 4: *In vivo* anti-tumor activity of CYAD-01 T-cells manufactured with the mAb or OptimAb process



Mice bearing an aggressive AML (THP-1) cell line received 3 weekly injections of 3 x 10^6 Mock, CYAD-01 mAb or CYAD-01 OptimAb T-cells per infusion. This dose was titrated for minimal activity of CYAD-01 mAb.

OPTIMIZED MANUFACTURING PROCESS

- With respect to the CYAD-01 product, the current manufacturing process (**mAb process**), used to date in the DEPLETHINK study, tends to produce more differentiated T-cells that are highly active in killing but less able to persist. There is an emerging view in the cell therapy field that T-cells with reduced differentiation may be more active in the therapeutic setting.
- Celyad has developed a new process named "**OptimAb**", which generates a higher frequency of less differentiated CYAD-01 T-cells as compared to previous process (**Figures 2 and 3**). The OptimAb manufactured cells also produce higher levels of interferon gamma upon challenge with tumor cells.
- In a preclinical model, the OptimAb-manufactured CYAD-01 showed much improved long-term anti-tumor activity as compared to the currently used mAb-manufactured CYAD-01 at the same dose of cells (**Figure 4**).

MAIN RESULTS

- **Study Status** (**Table 1**):
  - 9 patients have been enrolled so far in the two first DLs of the dose escalation segment of this Phase I study with the current mAb process,
  - The recruitment has been re-initiated at DL-2 with the **OptimAb-manufactured CYAD-01** for safety and cell kinetics comparability reasons (ongoing, data not shown).
- An encouraging safety profile was observed (**Table 2**) for all CYAD-01 infusions post CyFlu preconditioning chemotherapy. To note, at the 1st CYAD-01 infusion of the consolidation cycle ($3x10^9$ cells per infusion), 1 patient at DL-1 (T7) experienced a Grade (G) 4 cytokine release syndrome (CRS) and a G3 CAR T-cell-related encephalopathy syndrome (CRES) and 1 patient at DL-2 experienced a G3 CRS. All patients recovered with treatment including tocilizumab.
- No objective response has been observed at the first two DLs but 3/9 patients did not progress one month after the first CYAD-01 infusion and were eligible for the consolidation cycle.
- For the two first DLs evaluated with the mAb process, the CYAD-01 cell engraftment is dose-dependent (**Figure 1**), and the addition of the CyFlu as preconditioning induces a better time-averaged engraftment as compared to the CYAD-01 injected without preconditioning (**Figure 1** and THINK **poster 3826**).

CONCLUSIONS

- To date, the results demonstrate the **safety** of $1x10^8$ and $3x10^8$ **mAb-manufactured CYAD-01 cells**/infusion administered after cyclophosphamide/fludarabine preconditioning chemotherapy.
- The preconditioning regimen increases the **engraftment** of the CYAD-01 cells as compared to cells infused with no preconditioning, and, for the two first DLs evaluated, the CYAD-01 cell engraftment is dose-dependent.
- In a preclinical model, CYAD-01 produced with an optimized manufacturing process ("**OptimAb**") showed an improved long-term anti-tumor activity at the same dose as compared to the currently used process.
- The DEPLETHINK study has been reinitiated at DL-2 with the OptimAb-manufactured CYAD-01 product, which should help to increase expansion of the cells and favor long-term anti-tumor activity.
- In parallel, the OptimAb-manufactured CYAD-01 will be also evaluated without prior preconditioning therapy (**poster 3826**) and a **next-generation NKG2D CAR**, CYAD-02, which includes features further favoring the persistence of the CAR T-cells *in vivo*, will be evaluated into the CYCLE-1 Phase I study (NCT04167696) post preconditioning chemotherapy (**poster 3931**), both in the same patient population.

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- ASAH, EP, PL, MAH, DP, AS, IM and TK are Investigators on the DEPLETHINK trial. BD, TL,MSD, FB, CL, NB, FR and AF are employed by Celyad SA.
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