First Results from the Dose Escalation Segment of the Phase I Clinical Study Evaluating CYAD-02, an Optimized Non-Gene-Edited Engineered NKG2D CAR T-cell Product, in Relapsed or Refractory Acute Myeloid Leukemia and Myelodysplastic Syndrome Patients

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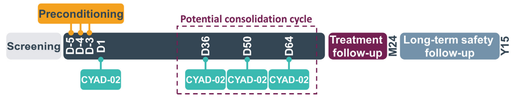
BACKGROUND

- Effective therapeutic options for patients with relapsed/refractory (r/r) acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are lacking.
- Chimeric antigen receptor (CAR) T cell therapy is delivering major clinical responses in B-cell malignancies but there are no validated CAR T cell targets in AML/MDS yet.
- CYAD-02 is based on an autologous NKG2D CAR product, CYAD-01, which has shown some initial signs of transient clinical activity as a monotherapy in r/r AML/MDS patients (**ASH 2019 – poster 3826**). However this activity was not enhanced by modifying the manufacturing process (OptimAb) or when combined with preconditioning chemotherapy (**poster 993**).
- CYAD-02 is a next-generation autologous CAR T cell product based on the fusion of the NKG2D receptor with CD3ζ. NKG2D binds eight different stress induced ligands (MICA/B, ULBP1-6) that are over-expressed by a large variety of malignancies including AML/MDS.
- Since preclinical studies have shown that the transient upregulation of NKG2D ligands MICA and MICB on activated CAR T-cells might decrease *in vivo* persistence of the cells (**Breman et al. Frontiers Immunol 2018**), **CYAD-02** uses a **non-gene editing approach** to silence the expression of MICA and MICB with the aim to increase persistence and potency of the NKG2D CAR T-cells.
- Co-expressing a MICA/B short hairpin (shRNA) with the NKG2D CAR and using the OptimAb manufacturing process results in a T-cell product which displays improved anti-tumor activity in preclinical models (**ASH 2019 – poster 3931**).
- The Phase 1 CYCLE-1 (**NCT04167696**) study was initiated to evaluate this next-generation CYAD-02 product post a preconditioning chemotherapy.
- The dose levels and schedule closely follow that of the DEPLETHINK study (**NCT03466320; poster 993**) to permit a comparison between the activity of CYAD-01 and CYAD-02.

CYCLE-1 STUDY

- The Phase 1 CYCLE-1 study evaluates a single infusion of CYAD-02 cells after non-myeloablative preconditioning chemotherapy in patients with r/r AML/MDS.
- The preconditioning chemotherapy consists of 300 mg/m² cyclophosphamide and 30 mg/m² fludarabine daily for 3 days (CyFlu).



- Dose escalation study with a 3+3 study design evaluates three dose levels (DL) of CYAD-02: 1x10^8, 3x10^8 and 1x10^9 total cells per infusion.
- A consolidation cycle with CYAD-02 given once every two weeks for three infusions without prior preconditioning chemotherapy is authorized in the absence of progressive disease after the first CYAD-02 infusion and no detectable CYAD-02 in the peripheral blood.
- Primary endpoint is the occurrence of dose-limiting toxicity (DLT). Key secondary endpoints include additional safety parameters, CYAD-02 cell kinetics, objective responses and duration of responses.

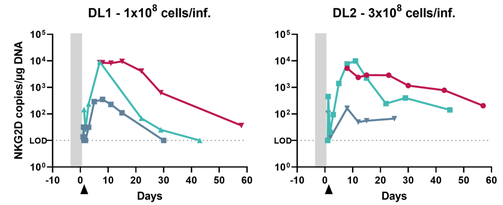
TABLES & FIGURES

Table 1: CYCLE-1 Study patient baseline characteristics

Study snapshot: 22 Oct 2020	DL1 1x10^8 N=3	DL2 3x10^8 N=3	DL3 1x10^9 N=1	All patients N = 7
Age (years): Mean (Range)	70.3 (66-79)	63.0 (41-74)	60	65.7 (41-79)
Gender: Male/Female	1/2	3/0	1/0	5/2
ECOG at screening (Grade 0/1)	0/3	1/2	0/1	1/6
Previous lines of therapies: Mean (Range)	1 (1-1)	3 (1-6)	2	2 (1-6)
Tumor type				
r/r Acute Myeloid Leukemia	1	1		2
r/r Myelodysplastic Syndrome	2	2	1	5
ELN 2017/R-IPSS Risk Stratification				
Favorable (AML)/Intermediate (MDS)	0/0	0/0	0/0	0/0
Intermediate (ALM)/High-Risk (MDS)	0/0	0/1	0/0	0/1
Adverse (AML)/Very High-Risk (MDS)	1/2	1/1	0/1	2/4
Bone marrow blasts (%) mean (range)	31.3 (14-63)	19.0 (4-28)	6	22.4 (4-63)
Peripheral blood blasts (%) mean (range)	11.1 (0-22.1)	7.3 (0-21)	0	7.9 (0-22.1)
Platelets (10^3/µL) mean (range)	75.0 (33-125)	57.0 (17-83)	100	70.9 (17-125)
ANC (10^3/µL) mean (range)	1.17 (0.15-2.37)	1.7 (0.5-2.9)	0.15	1.2 (0.15-2.9)

(data from uncleaned database)

Figure 1: CYAD-02 kinetics in the peripheral blood



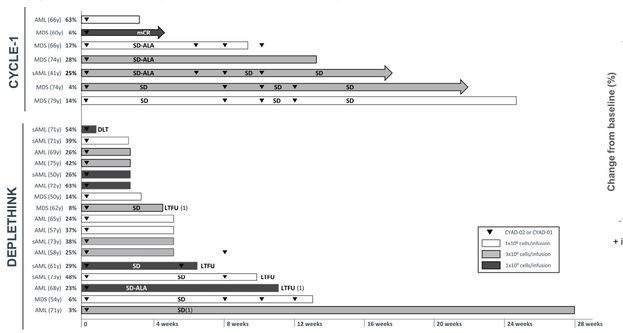
CYAD-02 cell kinetics was determined by digital droplet polymerase chain reaction on genomic DNA from PBMCs isolated from blood collected at pre-specified timepoints. Values below the limit of detection (LOD) were put at LOD (dotted line). Arrow indicates the CYAD-02 cell infusion. Gray bar indicates CyFlu preconditioning.

Table 2: Incidence of treatment-related adverse events (AEs) in patients infused with CYAD-02 (CYCLE-1 Study) and CYAD-01 OptimAb (DEPLETHINK Study)

Study snapshot: 22 Oct 2020	CYCLE-1 DL1 (1x10^8) INDUCTION / CONSOLIDATION N=3 (3 inf.)/ N=2 (6 inf.)			CYCLE-1 DL2 (3x10^8) INDUCTION / CONSOLIDATION N=3 (3 inf.)/ N=2 (6 inf.)			CYCLE-1 DL3 (1x10^9) INDUCTION / CONSOLIDATION N=1 (1 inf.)/ N=0 (0 inf.)			DEPLETHINK DL2 (3x10^8) INDUCTION / CONSOLIDATION N=3 (3 inf.)/ N=1 (1 inf.)			DEPLETHINK DL3 (1x10^9) INDUCTION / CONSOLIDATION N=4 (4 inf.)/ N=0 (0 inf.)		
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**	**All Grades**	**Grade 3**	**Grade 4**
Related AEs with highest grade per infusion	7/3	1/0	1/0	6/3	-	-	1/NA	1/NA	-	6/0	1/0		8/NA	4/NA	-
Acute respiratory failure	-	-	-	-	-	-	-	-	-	-	-	-	1/NA	-	-
Blood bilirubin increased	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Breath sounds abnormal	-	-	-	-	-	-	-	-	-	-	-	-	1/NA	-	-
Cytokine release syndrome	1/3	-	-	3/3	-	-	1/NA	1/NA	-	3/0	-	-	3/NA	1/NA	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	-	1/NA	-	-
Disseminated intravascular coagulation	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fatigue	-	-	-	2/0	-	-	-	-	-	-	-	-	-	-	-
Febrile neutropenia	1/0	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-
Headache	-	-	-	1/0	-	-	-	-	-	-	-	-	-	-	-
Hypotension	-	-	-	-	-	-	-	-	-	-	-	-	-	1/NA	-
Infusion reaction	1/0	-	1/0	-	-	-	-	-	-	-	-	-	-	-	-
Lymphocyte count decreased	-	-	-	-	-	-	-	-	-	-	1/0	-	-	-	-
Neurotoxicity	-	-	-	-	-	-	-	-	-	1/0	-	-	-	-	-
Oxygen saturation decreased	-	-	-	-	-	-	-	-	-	-	-	-	1/NA	-	-
Pulmonary oedema	-	-	-	-	-	-	-	-	-	-	-	-	-	1/NA	-
Pyrexia	-	-	-	-	-	-	-	-	-	2/0	-	-	1/NA	1/NA	-
Stomatitis	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weight decreased	1/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-

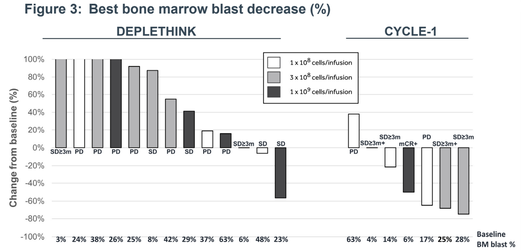
(data from uncleaned database) NA: not applicable (no patient with a consolidation cycle)

Figure 2: Clinical responses and duration of responses



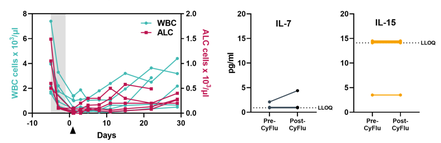
(Data from uncleaned database) Each bar represents one patient. Bar stopped at the first documented progression of the disease or when the patient was lost to follow-up (LTFU). ALA: anti-leukemic activity, defined as a decrease of at least 50% of the bone marrow blasts ; (s)AML: (secondary) acute myeloid leukemia; MDS: myelodysplastic syndrome LTFU: Lost to follow up; SD: Stable disease
(1) Patient not authorized to consolidation cycle due to CYAD-01 peripheral blood persistence

Figure 3: Best bone marrow blast decrease (%)



+ indicates patient is ongoing. (data from uncleaned database)

Figure 4: Lymphocyte count and cytokine modulation by the CyFlu chemotherapy in CYCLE-1



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MAIN RESULTS

- As of Oct 22, 2020, 7 patients (2 AML and 5 MDS) have been treated: 3 patients at DL1, 3 patients at DL2, 1 patient at DL3. The patient demographic and key baseline characteristics are outlined in **Table 1.**
- An encouraging safety profile was observed (**Table 2**) for all CYAD-02 infusions. One Grade (G) 4 infusion reaction and one G3 cytokine release syndrome (CRS) have been observed, both rapidly controlled with appropriate treatments.
- Clinical activity (**Figures 2 and 3**):
  - **CYCLE-1 study**: Of the 7 patients enrolled, 4 patients have presented a relevant bone marrow (BM) blast decrease, i.e., anti-leukemic activity (ALA), defined as decrease of at least 50% of the BM blasts. One of these patients, a r/r MDS patient, is presenting a marrow complete remission (mCR) per IWG criteria. Duration seems encouraging as 2 other patients are presenting a stable disease for more than 4 months (4m+ and 6m).
  - **DEPLETHINK study**: Of the 17 patients enrolled in the study, no objective responses were observed although 1 patient at the DL3 with the OptimAb process did show an ALA.
- Pharmacokinetics of the CYAD-02 cells in the peripheral blood (PB) (**Figure 1**):
  - CYAD-02 cells can be detected in the PB of patients soon after infusion. Peak concentrations (C_{max}) ranged from 163.9 to 9975.1 copies/µg of DNA (median=7256.1) and are observed within two weeks after infusion (median time to Cmax = 8 days, range 7-15).
  - CYAD-02 engraftment as measured by C_{max} and persistence of CYAD-02 two weeks after infusion are similar to what has been previously observed for CYAD-01 after CyFlu preconditioning chemotherapy (DEPLETHINK study, **poster 993**).
- Effect of the preconditioning on lymphocyte count and cytokine release (**Figure 4**):
  - CyFlu preconditioning induces deep lymphodepletion in AML/MDS patients as based on absolute lymphocyte count (ALC) and white blood cell (WBC) count.
  - CyFlu preconditioning does not induce significant alterations of cytokines, chemokines including homeostatic cytokines IL-7 and IL-15 in AML/MDS patients.

CONCLUSIONS

- Preliminary clinical activity data showed anti-leukemic activity in 50% of the r/r AML/MDS patients associated with an overall encouraging disease control. One objective mCR has been documented in the single patient enrolled so far at DL3.
- Cell products for the remaining recruited DL3 patients have been successfully produced.
- Initial observations of clinical activity observed in the CYCLE-1 study seems attributable to an increased potency of CYAD-02 given the apparent equivalent levels of cell engraftment seen in the CYCLE-1 and DEPLETHINK studies (similar CyFlu dosing).
- Despite the expected level of lymphodepletion induced by CyFlu preconditioning, there was no evidence of increased levels of homeostatic cytokines, a key driver of T cell expansion. This could be related to the impact of AML/MDS on bone marrow.
- Favorable safety profile for CYAD-02 observed in the CYCLE-1 Phase I study, to date.

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