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 r/r malignancies but those are not 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 3538). However, this activity was not enhanced by modifying the manufacturing process (OptimAb) or when combined with pre-conditioning 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 (MIC/A, ULBP/L) 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 MIC/M and MICA can activate CAR T-cells might decrease in vivo persistence of the cells (Brem et al., Frontiers Immunol. 2018). CYAD-02 uses a non-genetic editing approach (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 pre-conditioning 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 pre-conditioning chemotherapy.
- The dose levels and schedule closely follow that of the DEPLETHINK study (NCT03468230; 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 pre-conditioning chemotherapy in patients with r/r AML/MDS.
- The pre-conditioning chemotherapy consists of 300 mg/m² cyclophosphamide and 30 mg/m² fludarabine daily for 3 days (CyFlu).
- Dose escalation study with a 3+3 design evaluates three dose levels (DLs) of CYAD-02: 1x10⁷, 3x10⁷ and 1x10⁹ total cells per infusion.
- A consolidation cycle with CYAD-02 given once every two weeks for three infusions without prior pre-conditioning 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.

**MAIN RESULTS**

- As of Oct 22, 2020, 7 patients (3 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):
  - CYAD-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 (4r/m 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.
- Pharmacodynamics 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 (Cmax) 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 Cmax 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 hematopoietic 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 hematopoietic 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