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-chain, second-generation NKGD2 CAR comprising the full-length human natural killer group 2D (NKGD2) receptor fused to the intracellular domain of CD3ζ.

The NKGD2 receptor targets ligands IP38 in myeloid cells, 1 chain related proteins A (MICA) and B (MICB) and unique long 16 binding proteins (ULBP1-6) 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) express NKGD2 ligands which lead to preclinical models to the induction by CYAD-01 of a broader anti-tumor response beyond direct cancer cell killing.

In CYAD-01, being evaluated in relapsed/refractory (r/r) AML/MDS patients, the objective to define whether the optimal CYAD-01 treatment is with prior pre-conditioning chemotherapy DEPLETHINK study, here discussed or without any pre-treatment (see THNK study, poster 3823).

### DEPLETHINK STUDY

The open-label Phase I/II DEPLETHINK Study (NCT03663202) 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 500 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 NKGD2 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: 1x10⁶, 3x10⁶ and 1x10⁷ 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 (~1.5x10⁶/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 (late pre-conditioning: treatment administered 7 days (7T) or 3 days (3T) before CYAD-01 infusion) to evaluate any potential modulation of the CYAD-01 cell-engagement.
- Potential CYAD-01 consolidation cycle of 5 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
<table>
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<tr>
<th>Study patient</th>
<th>Total Number</th>
<th>ELN 2017/R-IPSS Risk Stratification for AML/MDS</th>
<th>Age Group (years)</th>
<th>Males/Females</th>
<th>AML/AML-MDS</th>
<th>MDS</th>
<th>MDS/MDS-MDS</th>
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<td>Age (years)</td>
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<td>16.0 (4.0-14.0)</td>
<td>22.0 (3.0-38.0)</td>
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<td>28.0 (6.0-48.0)</td>
<td>22.0 (3.0-38.0)</td>
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<td>Cytokine release syndrome</td>
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### 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 manufacturing process exploits the NKG2D ligand-interfering 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 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 1° CYAD-01 infusion of the consolidation cycle (3x10⁶ cells per infusion), patients enrolled with a Grade 0/1/2 cytokine release syndrome (CRS) and a GS CAR T-cell related engraftment syndrome (ERS) and 1 patient at DL 2 experienced a GS CRS. 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 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 THNK poster 3823.

### CONCLUSIONS

To date, the results demonstrate the safety of 1x10⁶ and 3x10⁶ 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 infusion alone with no preconditioning, and, for the first 2 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 3824) and a next-generation NKGD2 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 (NCT03410796) post preconditioning chemotherapy (poster 3823), both in the same patient population.

### ACKNOWLEDGEMENTS & DISCLAIMER

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AGAM, D’PE, HWA, D’SL, IM and T are employees of the Celyad SA and may hold potential conflicts of interest related to the Celyad’s CAR T-cell program.

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