NKG2D-based chimeric antigen receptor therapy induced remission in a relapsed/refractory acute myeloid leukemia patient

David A. Sallman,¹ Jason Brayer,¹ Elizabeth M. Sagatys,² Caroline Lonez,¹ Eytan Bremann,¹ Sophie Agaugué,³ Bikash Verma,⁴ David E. Gilham,³ Frédéric F. Lehmann³ and Marco L Davila⁵

¹Malignant Hematology and ²Hematopathology and Laboratory Medicine, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA; ³Celyad, SA, Mont-Saint-Guibert, Belgium; ⁴Celyad, SA, New York, NY, USA and ⁵Blood & Marrow Transplantation and Cellular Immunotherapy, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

DAS and JB contributed equally to this work.

Correspondence: David.Sallman@moffitt.org or Marco.Davila@moffitt.org
Supplementary information for:

NKG2D-based chimeric antigen receptor therapy induced remission in a relapsed/refractory acute myeloid leukemia patient.

Authors and affiliations

David A. Sallman\textsuperscript{1*}, Jason Brayer\textsuperscript{1*}, Elizabeth M. Sagatys\textsuperscript{2}, Caroline Lonez\textsuperscript{3}, Eytan Breman\textsuperscript{3}, Sophie Agaugué\textsuperscript{3}, Bikash Verma\textsuperscript{4}, David E. Gilham\textsuperscript{3}, Frédéric F. Lehmann\textsuperscript{3}, Marco L Davila\textsuperscript{5}

\textsuperscript{1}Malignant Hematology and \textsuperscript{2}Hematopathology and Laboratory Medicine, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

\textsuperscript{3}Celyad, SA, Mont-Saint-Guibert, Belgium

\textsuperscript{4}Celyad, SA, New York, NY, USA

\textsuperscript{5}Blood & Marrow Transplantation and Cellular Immunotherapy, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

*These authors contributed equally to this work.
**Fig. S1: Patient’s CYAD-01 phenotype and in vitro functionality.** (A) A sample of the infusion product was analyzed for cell subset composition and memory phenotype. CD3⁺ viable singlet cells showed a high CD8 preponderance with both CD4 and CD8 subsets possessing a majority of CD62Llo phenotype. As shown in the CD8 subset, both CD62Lhi and CD62Llo populations were CD95⁺ and CD28lo. Together this suggests the infusion product to be composed primarily of CD8⁺ T cells that possess an effector memory phenotype. 88% of the CD4⁺ T cells and 94% of the CD8⁺ T cells were NKG2D+. (B) Patient’s CYAD-01 product was incubated in the presence or absence of NKG2D blocking antibody (CD314 Ab) (light and dark grey bars, respectively) with PANC-1 and K562 cancer cells (at a 1:1 ratio). After 24h of incubation, supernatants were harvested and analyzed for IFN-γ secretion. Dotted line represents the limit of detection (LOD). Each bar represents the mean and SD of one experiment conducted at least in duplicate, ****: p < 0.001. (C) Patient’s CYAD-01 cells were cultured at a 1:1 ratio with PANC-1 cells in the presence or absence of CD314 blocking Ab. After a 20h
incubation the CYAD-01 cells were washed away and the remaining PANC-1 cells stained with AlamarBlue to quantify the remaining proliferating PANC-1 cells. Each bar represents one experiment conducted in triplicate with the corresponding SD, *: p < 0.05.
Fig. S2: Immunohistochemistry analysis of NKG2D ligand expression in bone marrow sample taken before first CYAD-01 injection. One BM biopsy was subject to a range of IHC staining protocols specific for the following NKG2DL: MICA/MICB, ULBP1, ULBP2/5/6, ULBP3. In addition, a sample of each was prepared applying standard hematoxylin and eosin (H&E) staining (data not shown). All samples were examined and graded by light microscopy. Representative stainings of ULBP1 (A), ULBP2/5/6 (B) and ULBP3 (C). It has to be noted that ULBP2/5/6 are predominantly stained in the nucleus and that ULBP1, ULBP3 and MICA/B (Fig. 2B) display cytoplasmic staining as well.
Supplementary Methods

Study THINK design

The THINK (Therapeutic Immunotherapy with NKR-2) trial is an open-label Phase I study which primarily aims to assess the safety and clinical activity of the CYAD-01 treatment administered three times at 2 weeks intervals between each administration without prior lymphodepleting chemotherapy in patients with refractory or relapsing malignancies, including patients with metastatic or locally advanced colorectal cancer, urothelial carcinoma, triple-negative breast cancer, pancreatic cancer, recurrent epithelial ovarian and fallopian tube carcinoma, AML/MDS or MM. The study is split into two segments; a dose escalation segment evaluating three dose-levels ($3 \times 10^8$, $1 \times 10^9$ and $3 \times 10^9$ cells/injection) to determine the recommended dose of CYAD-01 cells and an expansion phase to investigate the clinical activity across multiple tumor indications while extending the safety study.

Manufacture of cell products

CYAD-01 (previously known as NKR-2) refers to the viable cell population obtained after retroviral transduction of autologous T-cells with the NKG2D-based CAR. CYAD-01 will be supplied cryopreserved in bags containing a T-cell dose in accordance with the dose-level which is to be administered.

Characterization of the patient CYAD-01

CYAD-01 identity (% NKG2D on CD4+, CD4+CD8+ and CD8+ T cells), purity (% viable CD3+) and viability (with 7AAD dye) are assessed by flow cytometry. Cell yield is assessed by cell counting (excluding Trypan blue). In vitro product functionality/potency is evaluated by assessment of IFN-γ secretion via ELISA and metabolic activity of tumor cells via Alamar Blue assay upon co-culture of CYAD-01 with NKG2D ligand-expressing tumor cells (Panc-1 cells...
and/or K562). NKR-2 microbiological safety was confirmed by absence of microbiological
growth, assessed by BactAlert, absence of mycoplasms, assessed by qPCR based MycoTool
assay, and compliant endotoxin level (<8.67 EU/ml), assessed by PTS EndoSafe LAL assay.
Patient CYAD-01 met the product specifications. CYAD-01 phenotype identity (90% NKG2D⁺), purity (95% CD3⁺), viability (86%) and cell yield (300 x 10⁶ cells), evidenced a viable, highly pure CD3⁺, NKG2D⁺ product. Viral vector safety was evaluated by Vector copy number (VCN) and Replication copy number (RCR) qPCR-based assays. In vitro product functionality/potency was confirmed by IFN-γ secretion and efficient cytotoxicity effect in response to NKG2DL on tumor cells.