CYAD-01, an autologous NKG2D-based CAR T-cell therapy, in relapsed or refractory acute myeloid leukaemia and myelodysplastic syndromes or multiple myeloma (THINK): haematological cohorts of the dose escalation segment of a phase 1 trial

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Summary

Background CYAD-01 is an autologous chimeric antigen receptor (CAR) T-cell product based on the natural killer (NK) group 2D (NKG2D) receptor, which binds eight ligands that are overexpressed in a wide range of haematological malignancies but are largely absent on non-neoplastic cells. Initial clinical evaluation of a single infusion of CYAD-01 at a low dose in patients with relapsed or refractory acute myeloid leukaemia, myelodysplastic syndromes, and multiple myeloma supported the feasibility of the approach and prompted further evaluation of CYAD-01. The aim of the present study was to determine the safety and recommended phase 2 dosing of CYAD-01 administered without preconditioning or bridging chemotherapy.

Methods The multicentre THINK study was an open-label, dose-escalation, phase 1 study for patients with relapsed or refractory acute myeloid leukaemia, myelodysplastic syndromes, or multiple myeloma, after at least one previous line of therapy. Patients were recruited from five hospitals in the USA and Belgium. The dose-escalation segment evaluated three dose levels: 3×10^8 (dose level one), 1×10^9 (dose level two), and 3×10^9 (dose level three) cells per infusion with a 3+3 Fibonacci study design using a schedule of three infusions at 2-week intervals followed by potential consolidation treatment consisting of three additional infusions. The occurrence of dose-limiting toxicities post-CYAD-01 infusion was assessed as the primary endpoint in the total treated patient population. The trial was registered with ClinicalTrials.gov, NCT03018405, and EudraCT, 2016-003312-12, and has been completed.

Findings Between Feb 6, 2017, and Oct 9, 2018, 25 patients were registered in the haematological dose-escalation segment. Seven patients had manufacturing failure for insufficient yield and two had screening failure. 16 patients were treated with CYAD-01 (three with multiple myeloma and three with acute myeloid leukaemia at dose level one; three with acute myeloid leukaemia at dose level two; and six with acute myeloid leukaemia and one with myelodysplastic syndromes at dose level three). Median follow-up was 118 days (IQR 46–180). Seven patients (44%) had grade 3 or 4 treatment-related adverse events. In total, five patients (31%) had grade 3 or 4 cytokine release syndrome across all dose levels. One dose-limiting toxicity of cytokine release syndrome was reported at dose level three. No treatment-related deaths occurred, and the maximum tolerated dose was not reached. Three (25%) of 12 evaluable patients with relapsed or refractory acute myeloid leukaemia or myelodysplastic syndromes had an objective response. Among responders, two patients with acute myeloid leukaemia proceeded to allogeneic haematopoietic stem-cell transplantation (HSCT) after CYAD-01 treatment, with durable ongoing remissions (5 and 61 months).

Interpretation Treatment with a multiple CYAD-01 infusion schedule without preconditioning is well tolerated and shows anti-leukaemic activity, although without durability outside of patients bridged to allogeneic HSCT. These phase 1 data support the proof-of-concept of targeting NKG2D ligands by CAR T-cell therapy. Further clinical studies with NKG2D-based CAR T-cells are warranted, potentially via combinatorial antigen targeted approaches, to improve anti-tumour activity.

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Introduction

Autologous chimeric antigen receptor (CAR) T-cell therapies have shown durable clinical benefit for patients with B-lymphoid malignancies or multiple myeloma.[1,2] However, for other malignancies, CAR T-cell therapy has yet to show similar clinical efficacy. In the case of acute

Research in context

Evidence before this study

We searched PubMed for clinical reports published in English on the use of chimeric antigen receptor (CAR) T-cells for acute myeloid leukaemia. Search terms included "acute myeloid leukaemia", "chimeric antigen receptors" or "CAR-T". Most adults with acute myeloid leukaemia or myelodysplastic syndromes have refractory or relapsed disease after treatment due to the presence of residual acute myeloid leukaemia cells and will ultimately die with the current standard of care. Since the 2000s, immunotherapy has emerged as another therapeutic option for various types of cancer but a paucity of a specific tumour antigen expressed only on blasts but not on stem cells, and the strong immunosuppressive and complex microenvironment, have limited the success of those approaches in acute myeloid leukaemia and myelodysplastic syndromes. CYAD-01 is an autologous T-cell product engineered to express an NKG2D-based CAR, which recognises eight ligands from two distinct families expressed by most malignant cell types and not present on healthy normal cells, including haematopoietic stem cells and progenitor cells. Preclinical data evidenced that, apart from efficient tumour cell killing, CYAD-01 also targets the tumour microenvironment and tumour vasculature, while inducing a long-term adaptive host immune response. These data supported the hypothesis that CYAD-01 could provide a multipronged approach to tumour eradication, limiting the risk of relapse due to antigen loss by targeting multiple ligands and controlling any minimal residual disease by the induction of a patient anti-tumour adaptive immune response.

Added value of this study

The THINK study is one of the first completed dose-escalation CAR T-cell studies in relapsed or refractory acute myeloid leukaemia or myelodysplastic syndromes. Unlike all other studies evaluating CAR T-cell therapy, the THINK study evaluated multiple infusions of CYAD-01 as a stand-alone therapy (ie, without previous bridging or preconditioning chemotherapy). This feature is of particular interest considering the median older age and the poorer general condition of patients with relapsed or refractory acute myeloid leukaemia or myelodysplastic syndromes at diagnosis.

Implications of all the available evidence

The encouraging data from this study in a difficult-to-treat patient population support the further clinical development of CYAD-01 or other NKG2D-based CAR T-cell therapies, including dual-targeting CAR T-cell therapy, although the need to improve this treatment is clear. If confirmed and optimised in a larger patient population, NKG2D-based CAR T-cell products could become a new treatment option for patients with relapsed or refractory acute myeloid leukaemia or myelodysplastic syndromes.

myeloid leukaemia and myelodysplastic syndromes, aside from the high genetic and epigenetic heterogeneity, the paucity of blast and leukaemia stem cell-specific targets has been a challenge for the standard single chain variable fragment-based targeting approaches used in CAR T-cell therapy.[3] The targets typically explored—eg, CD33 and CD123 antigens—tend to be expressed on haematopoietic stem and progenitor cells, with high potential for therapy-limiting, on-target, off-tumour toxicity, although recent reports showed manageable toxicity but with unclear efficacy that could be related to heterogeneous antigen expression.[4–6] Approaches by which CAR T-cells could be regulated by reliable and fast-acting on–off switches, to avoid acute and long-term toxicity usually associated with continuous activation of CAR-T cells, are also currently being developed and early results have provided initial clinical proof of concept and preliminary activity.[7] However, acute myeloid leukaemia and myelodysplastic syndromes are characterised by a highly immunosuppressive micro-environment composed of, among others, myeloid-derived suppressor cells and immunosuppressive regulatory T-cells, which can drive CAR T-cell exhaustion.[8,9] The persistent poor outcomes of adult patients with relapsed or refractory acute myeloid leukaemia and myelodysplastic syndromes, despite various new treatment strategies, underscores the need for the development and implementation of novel agents with a different mechanism of action.[10]

The natural killer (NK) group 2D (NKG2D) receptor is a primary activating receptor for NK cells that binds eight stress-induced ligands belonging to two families, the MHC class I chain-related proteins A and B, and the structurally diverse unique long 16 binding proteins 1 to 6.[11] Malignant cells from both solid and haematological cancers, including acute myeloid leukaemia and myelodysplastic syndromes blasts, and multiple myeloma cells, express several NKG2D ligands, while their expression in healthy non-neoplastic cells (including stem cells) remains largely absent or undetectable, making them promising targets for CAR T-cell approaches.[12] Nevertheless, NKG2D ligand expression can be induced by multiple stress conditions such as viral infection, oxidative or thermal stress, DNA damaging agents, tissue damage, heat shock, or inflammation, in addition to malignant transformation.[13] Therefore, treatment with chemotherapy before CAR T-cell treatment could potentially result in the induction of NKG2D ligands on healthy tissues, increasing the risk of toxicity, although this has not been observed in clinical trials to date.[14]

CYAD-01 is an autologous CAR T-cell product engineered to express a NKG2D-based CAR, consisting of the full-length human NKG2D receptor fused with the human CD3ξ signalling domain, which interacts with the endogenous adaptor molecule DNAX-activating protein of 10 kDa (DAP10) to mediate signal transduction and cellular activation upon ligand recognition.[15] Therefore,

although the CAR format suggests a first generation configuration, the CYAD-01 works more like a second generation CAR via its interaction with DAP10. Preclinical studies have shown that CYAD-01 can induce long-term anti-tumour activity (>225 days) in syngeneic models in the absence of preconditioning,[16] with improved efficacy following multiple administrations.[15,17] Importantly, CYAD-01 also targeted tumour neovasculature,[18] tumour resident myeloid-derived suppressor cells, and regulatory T-cells within the tumour environment in vivo,[19] which induced an effective endogenous anti-tumour adaptive immune response.[12]

Preclinical studies supported a first-in-human clinical study (CM-CS1; NCT02203825) evaluating a single infusion of low-dose CYAD-01 in the absence of pre-conditioning chemotherapy.[20] The CM-CS1 study, in conjunction with preclinical data, suggested that increased dosing and multiple infusions of CYAD-01 should be pursued. Consequently, the THINK study was initiated to evaluate the safety of multiple infusions of CYAD-01 administered every 2 weeks in the absence of pre-conditioning chemotherapy. The study included patients with both haematological malignancies and solid tumours, in two separate segments.[21] The aim of the present study was to determine the safety and recommended phase 2 dosing of CYAD-01 administered without preconditioning or bridging chemotherapy. In this Article, we focus upon the haematological dose-escalation segment which included patients with relapsed or refractory acute myeloid leukaemia, myelodysplastic syndromes, or multiple myeloma.

Methods

Study design

The THINK study was a multicentre, open-label, dose-escalation phase 1 study. The dose-escalation segment evaluated three doses of CYAD-01 (dose level one: 3×10^8 total cells per each infusion; dose level two: 1×10^9 total cells per each infusion; and dose level three: 3×10^9 total cells per each infusion, to be adjusted per body weight for patients weighing ≤65 kg at $4{\cdot}6\times10^6$ CYAD-01 per kg, $1{\cdot}5\times10^7$ CYAD-01 per kg, and $4{\cdot}6\times10^7$ CYAD-01 per kg, respectively) using a Fibonacci 3+3 design to determine the recommended phase-2 dose of CYAD-01 treatment based on the occurrence of dose-limiting toxicities.[21] Patients who discontinued treatment before completing the visit at day 43, in the absence of dose-limiting toxicity, were considered unevaluable and were replaced by a new patient at the same dose-level. Another treatment schedule consisting of three CYAD-01 infusions, once per week, during first cycle of treatment (without preconditioning) was also proposed in two separate cohorts, with the aim to maintain cell persistence through regular infusions. However, early data did not show an improvement in clinical outcome as compared with the 2-weeks interval schedule and this approach was not further pursued.

At the initiation of the study, the CYAD-01 cells were manufactured with the LY process,[22] which has a high manufacturing failure rate, probably due to NKG2D ligand expression on CAR T-cells. Therefore, the process was changed to add a NKG2D-blocking antibody (referred to as monoclonal antibody process) with improved results (appendix pp 7–8).

See Online for appendix

The study was done in five hospitals in the USA and in Belgium (appendix p 8) in accordance with the Declaration of Helsinki and adhered to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use guideline for Good Clinical Practice. All participants provided written informed consent before registration in the study. The protocol and patient-informed consent (original versions and all subsequent amendments up to version 6.0) were approved by the relevant institutional review board or independent ethics committee before study initiation at each site.

Participants

Participants were 18 years or older, with an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or lower. Eligible patients had relapsed or refractory disease after previous treatment defined as either (1) acute myeloid leukaemia presenting at least 5% blasts in bone marrow or in peripheral blood after at least one previous therapy; (2) myelodysplastic syndromes with at least 5% blasts in bone marrow, at least 2% blasts in peripheral blood, or *TP53* mutation after at least four cycles of azacitidine or decitabine; or (3) multiple myeloma after at least three previous combination therapies. Patients with acute myeloid leukaemia also had to have less than 15 000 per µL absolute peripheral blast count at baseline. No documented NKG2D ligand expression was required at baseline for eligibility, although this was assessed retrospectively as a correlative biomarker analysis. Women of child-bearing potential (ie, those who had not had a hysterectomy or ovariectomy, and who were pre-menopausal) and men had to agree to use effective contraception before, during, and for at least 2 months after the last study treatment administration.

Additional baseline assessments required for eligibility, as assessed by standard laboratory criteria and spirometry, were adequate hepatic and renal functions, a left ventricular ejection fraction of 40% or more and a forced expiratory volume in 1 s (FEV_1) to forced vital capacity ratio of 0·7 or higher, with FEV_1 at least 50% predicted. Patients were excluded if they had any serious uncontrolled medical disorder, persistent toxicities greater than or equal to Common Terminology Criteria for Adverse Events (CTCAE) grade 2 caused by previous cancer therapy, active infections, previous allogeneic haematopoietic stem-cell transplantation (HSCT) or genetically modified T-cell therapies were the main exclusion criteria (appendix pp 8–11). Patients who had received any cancer therapy (investigational agent or not,

including bridging therapy) or radiotherapy within 2 weeks before the planned day for apheresis, and within 3 weeks before the planned CYAD-01 infusion, were also excluded from the study under protocol version 1.0 (dated Aug 16, 2016) and version 5.0 (dated Aug 17, 2018) under which the presented patients were enrolled.

Although patients with multiple myeloma were still eligible per protocol after the implementation of the monoclonal antibody process, the initial manufacturing failure rate at dose level one and the progressive availability of other treatment alternatives for a similar patient population during the course of the study strongly affected the recruitment of this indication in the study.

Patients were not randomly assigned to treatment group. For dose level two and beyond, only patients with acute myeloid leukaemia or myelodysplastic syndromes were recruited upon investigator's decision and based on the higher rate of manufacturing failures in the multiple myeloma indication.

Procedures

Following informed consent, patients underwent apheresis at the clinical centres to isolate peripheral blood mononuclear cells for manufacturing of CYAD-01. The fresh apheresis was transported to Celyad Oncology's cell manufacturing unit (Mont-Saint-Guibert, Belgium) to produce the CAR T-cells at the dose level assigned. Three cryopreserved CYAD-01 bags (from the same apheresis and corresponding to the three planned infusions) were shipped to the clinical centres for thawing, reconstitution, and infusion.

Patients were scheduled to receive three CYAD-01 infusions by intravenous administration (at 10–15 min per 100 mL) every 2 weeks, at the assigned dose level. The first CYAD-01 infusion was administered on day one, 3 weeks (within 3 days) after the apheresis date. For patients not in progressive disease (ie, with stable disease or better response) at the first or second disease assessment (ie, day 29 or 57), a potential second cycle of three infusions every 2 weeks starting at the earliest 1 month after the third CYAD-01 infusion was proposed and implemented during dose level 2 and dose level 3 of the study. The rationale for the multiple infusion schedule with 2-week intervals was based on in-vivo data, which showed that a treatment regimen with a 2-week interval between injections had improved activity compared with a weekly interval.[19] Furthermore, a 2-week interval between infusions provided a window to monitor the patients for toxicities that might have been attributable to CYAD-01. Patients who had either (1) disease progression requiring new therapeutic approach; (2) any intolerable adverse event; (3) any confirmed dose-limiting toxicity following the previous CYAD-01 infusion; (4) treatment with any investigational or non-registered product, or anti-cancer therapy other than CYAD-01 administration; or (5) any medical condition requiring chronic treatment, were required to discontinue the study treatment administration. Systemic corticosteroids and other immunosuppressive drugs were not authorised during treatment unless in specific conditions including when used for counteracting an adverse event (eg, high risk of severe cytokine release syndrome [CRS], CAR-T-cell-related encephalopathy syndrome [CRES], or those with persistent CRS despite anti-interleukin [IL]-6 therapy). Steroids were also considered as a prevention strategy if a patient had a significant past CRS event.

Disease assessment in peripheral blood and bone marrow was done at baseline, then on days 29, and 57 and months 3, 6, 9, 12, 18, and 24 after the first infusion. Responses were assessed by the investigators according to the 2017 European Leukaemia Net recommendations for response criteria in acute myeloid leukaemia,[23] the 2006 International Working Group consensus criteria for myelodysplastic syndromes,[24] or the International Myeloma Working Group consensus criteria for multiple myeloma.[25] Blood, serum, and bone marrow samples were collected throughout treatment administration and follow-up phases for posthoc biomarker analyses (CYAD-01 transgene levels, cytokine and chemokine concentrations, and NKG2D ligand expression; appendix p 7).

Severity of adverse events was graded according to the National Cancer Institute's CTCAE (version 5.0) and evaluated at each visit. Severity of CRS and CRES was graded by the guidelines from the CAR-T-cell therapy-associated toxicity (CARTOX) working group.[26]

Outcomes

The primary endpoint of this study was the occurrence of dose-limiting toxicities at any time after initiation of the first CYAD-01 infusion up to 14 days after the third CYAD-01 administration (42 days). Dose-limiting toxicity was defined as a toxicity at least possibly related to the CYAD-01 treatment including (1) any CTCAE or CRS or CRES fatal (grade 5) toxicity not due to the underlying malignancy; (2) any CTCAE grade 4 study treatment-related toxicity that cannot be controlled to at most grade 2 within 7 days with appropriate treatment; (3) any grade 3 or worse CRS or CRES that cannot be controlled to at most grade 2 within 72 h with appropriate treatment; (4) any CTCAE grade 3 or worse allergic reactions related to the CYAD-01 infusion that cannot be controlled to at most grade 1 within 24 h with appropriate treatment; or (5) any CTCAE grade 4 haematological study treatment-related toxicity that cannot be controlled to at most grade 2 within 28 days with appropriate treatment.

Secondary endpoints were the (1) occurrence of adverse events and serious adverse events during study treatment; (2) occurrence of objective responses, including duration of response for patients who have objective responses; and (3) the overall survival and progression-free survival at 2 years. Objective response was defined as either

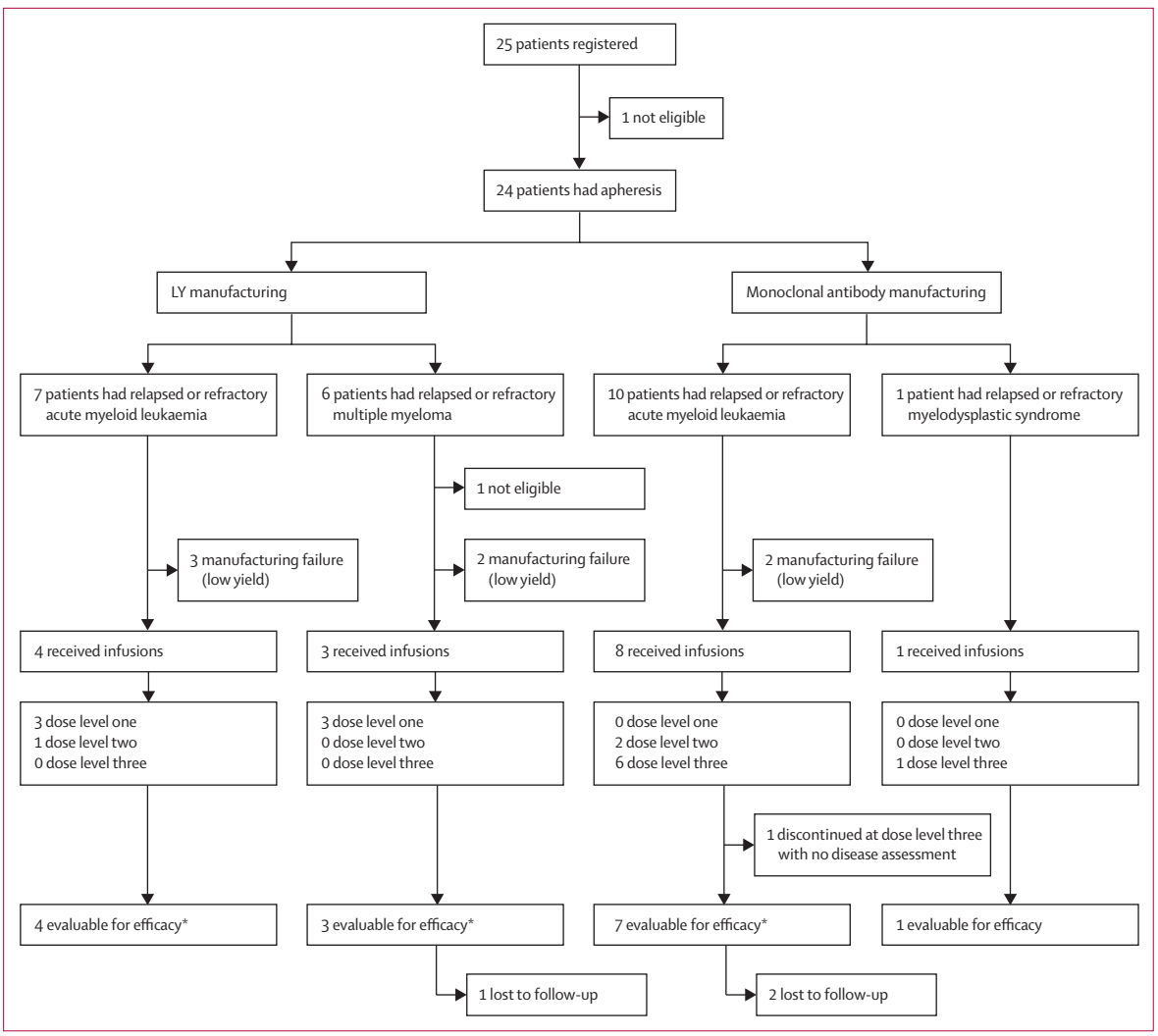

Figure 1: **Study profile**
Dose level one=3×10^8 cells per infusion. Dose level two=1×10^9 cells per infusion. Dose level three=3×10^9 cells per infusion. *One patient discontinued before the day 22 bone marrow evaluation but was considered still evaluable because of evident disease progression.

complete remission (with or without incomplete or partial haematological recovery), marrow complete remission, or partial remission. The mandatory tertiary endpoint was cellular kinetics of CYAD-01 cells in the peripheral blood.

Post-hoc biomarkers analyses were purely exploratory and included serum cytokines or chemokines and NKG2D ligand expression in tumour samples (appendix p 7).

These outcomes were centrally assessed by Celyad Oncology or by designated biomarker service providers.

Statistical analysis

All statistical analyses were primarily descriptive. Data were analysed with SAS (version 9.4) software. For biomarkers and cellular kinetics, GraphPad Prism software (version 9.4.1) was used. Based on a standard 3+3 design,[27] and assuming no occurrence of dose-limiting toxicity or replacement of patients, a sample size of nine participants was planned in the dose-escalation segment evaluating three CYAD-01 dose levels. When a patient discontinued treatment before completing the visit at day 43 and before having a dose-limiting toxicity, they were to be considered unevaluable and replaced by a new patient at the same dose level.

Baseline demographics and disease characteristics were summarised overall and by dose level. Safety statistics including dose-limiting toxicity numbers and summaries of adverse events and severe adverse events, were assessed on the basis of the total treated population (ie, all patients who received at least one CYAD-01 infusion). Adverse events reported were limited to those occurring within 28 days of the last CYAD-01 infusion. For the clinical activity analyses, the overall objective responses rate, median overall survival, and progression-free survival along with their 95% CIs, were calculated. As post-treatment disease assessment was done on

	Dose level one (n=6)	Dose level two (n=3)	Dose level three (n=7)	Untreated (n=9)
Age (years)	65 (55–73)	79 (60–83)	65 (48–72)	58 (53–60)
Age <65 years				
n	3 (50%)	1 (33%)	3 (43%)	7 (78%)
Median	55 (52–60)	60	48 (29–59)	57 (53–59)
Age ≥65 years				
n	3 (50%)	2 (67%)	4 (57%)	2 (22%)
Median	73 (70–79)	81 (79–83)	70 (67–76)	72 (70–73)
Sex				
Male	5 (83%)	2 (67%)	6 (86%)	4 (44%)
Female	1 (17%)	1 (33%)	1 (14%)	5 (56%)
ECOG performance score				
Grade 0	2 (33%)	1 (33%)	3 (43%)	2 (22%)
Grade 1	4 (67%)	1 (33%)	3 (43%)	7 (78%)
Grade 2	0	1 (33%)	1 (14%)	0
Haematological malignancy type				
Relapsed or refractory multiple myeloma	3 (50%)	0	0	3 (33%)
Relapsed or refractory myelodysplastic syndrome	0	0	1 (14%)	0
Relapsed or refractory acute myeloid leukaemia	3 (50%)	3 (100%)	6 (86%)	6 (67%)
De novo	2 (33%)	1 (67%)	3 (43%)	3 (33%)
Acute myeloid leukaemia myelodysplasia-related changes‡	1 (17%)	2 (67%)	3 (43%)	1 (11%)
Acute myeloid leukaemia secondary to myelodysplastic syndrome‡	1 (17%)	0	1 (14%)	1 (11%)
Missing data	0	0	0	2 (22%)
ELN 2017 risk classification by genetics for acute myeloid leukaemia				
Favourable	0	0	0	..
Intermediate	1 (17%)	1 (33%)	0	..
Adverse	2 (33%)	0	0	..
Unknown or missing	0	2 (67%)	4 (57%)	..
IPSS-R risk category for myelodysplastic syndrome				
Intermediate risk	..	..	1 (14%)	..
High risk	..	..	0	..
Very high risk	..	..	0	..
Acute myeloid leukaemia or myelodysplastic syndrome disease status at screening				
Bone marrow blasts (%)	10% (6–21)	24% (10–48)	12% (4–20)	..
Peripheral blasts				
Median	1·5% (0·0–4·5)	9·9% (0·0–20·0)	3·7% (0·0–14·4)	30·4% (2·0–56·0)
n	3 (50%)	3 (100%)	7 (100%)	5 (56%)
Platelets (10^3 cells per μL)	116 (8–296)	77 (15–131)	68 (3–188)	26·5 (19–284)
ANC (10^3 cells per μL)	1·9 (0·0–5·4)	1·3 (0·1–2·8)	1·0 (0·1–2·3)	0·7 (0·0–18·8)
NKG2D ligand in bone marrow blast expression at baseline				
MICA/B				
Median	85% (75–85)	53% (50–55)	100% (75–100)	..
n	3 (50%)	2 (67%)	3 (43%)	..
ULBP1				
Median	5% (5–55)	13% (0–25)	5% (NA)	..
n	3 (50%)	2 (67%)	3 (43%)	..
ULBP2/5/6				
Median	20% (15–30)	35% (10–60)	5% (0–50)	..
n	3 (50%)	2 (67%)	3 (43%)	..
ULBP3				
Median	35% (30–60)	22% (15–50)	25% (5–25)	..
n	3 (50%)	2 (67%)	3 (43%)	..

(Table 1 continues on next page)

	Dose level one (n=6)	Dose level two (n=3)	Dose level three (n=7)	Untreated (n=9)
(Continued from previous page)				
Previous lines of treatment				
1	2 (33%)	2 (67%)	3 (43%)	..
2	1 (17%)	0	3 (43%)	..
≥3	3 (50%)*	1 (33%)	1 (14%)	..
Median time from last chemotherapy treatment to apheresis in days	52 (17–76)	38 (22–48)	67 (44–128)	141 (57–257)
Median follow-up in days	134 (115–302)	52 (18–196)	102 (39–164)	14 (12–22)†

Data are median (IQR) or n (%). .. indicates that data were not collected as patient did not attend further visits. ANC=absolute neutrophil count. ELN=European Leukaemia Net. NA=not applicable as all results were the same. R-IPSS=Revised International Prognostic Scoring System. *All patients with multiple myeloma. †For untreated patients, the first and last dates of their screen, apheresis, or death dates were used for duration calculation. ‡Patients can be classified as having both acute myeloid leukaemia myelodysplasia-related changes and acute myeloid leukaemia secondary to myelodysplastic syndrome

Table 1: **Baseline characteristics in total population**

day 29, only patients who received three total infusions were included in the activity analysis.

Patients with multiple myeloma were stratified from those with acute myeloid leukaemia and myelodysplastic syndrome for the futility analysis for study continuation. Only two patients with multiple myeloma were evaluable, so no analysis specific to this disease was done.

This study is registered with ClinicalTrials.gov, NCT03018405, and EudraCT, 2016-003312-12.

Role of the funding source

The study was funded by the sponsor, Celyad Oncology, which was involved in study design, data collection, data analysis, and data interpretation, provided the study drug, and approved the final version of the manuscript for publication in conjunction with the authors.

Results

Between Feb 6, 2017, and Oct 9, 2018, 25 patients were registered in the haematological dose escalation segment (19 people with acute myeloid leukaemia or myelodysplastic syndromes and six people with multiple myeloma; figure 1). Of these, one patient with acute myeloid leukaemia had eligibility failure (ie, tumour progression) before apheresis, ten patients (six with acute myeloid leukaemia and four with multiple myeloma) had manufacturing failure (ie, low yield of cells) and one person with multiple myeloma had eligibility failure post-apheresis (ie, progression with CNS involvement). When possible, in case of low yield of cells not allowing infusion of the patient at the original intended dose, patients were offered infusion at the previous dose level. For this reason, three patients (one with acute myeloid leukaemia and two with multiple myeloma) for whom the yield did not allow for the three planned infusions at dose level two were included in the dose level one cohort. Overall, seven (30%) of 23 patients had full manufacturing failure (ie, too few cells for dose level one). All participants with acute myeloid leukaemia who had at least 30% peripheral blasts at baseline had manufacturing failure. The composition of the CAR T-cell products for the 13 treated patients with acute myeloid leukaemia or myelodysplastic syndromes are available in the appendix (p 2).

16 patients received at least one CYAD-01 infusion (three patients with multiple myeloma and three with acute myeloid leukaemia at dose level one, three with acute myeloid leukaemia at dose level two, and six with acute myeloid leukaemia and one with myelodysplastic syndromes at dose level three; appendix p 1). Median time from apheresis to first CYAD-01 infusion was 24 days (n=16, IQR 22–27). All infusions were done at least 29 months before the data cutoff of July 16, 2021. No patient received bridging therapy between apheresis and the first CYAD-01 infusion nor had previous allogeneic HSCT. Baseline demographics and clinical characteristics for the total treated patient population and untreated patients are shown in table 1. The median age was 67 (IQR 57–76). The median percentage of bone marrow blasts at baseline in patients with acute myeloid leukaemia or myelodysplastic syndromes was 12% (range 4–48%). Nine (69%) of 13 patients with acute myeloid leukaemia or myelodysplastic syndromes had at least two previous lines of therapy (median number of previous lines of therapies=1, IQR 1–2). Median follow-up was 118 days (IQR 46–180).

Two protocol deviations pertaining to investigational product preparation were reported for two participants but were considered as not substantially affecting data analysis: for one patient (referred to as AML7), the reconstitution time of the product was longer than allowed per protocol at third infusion (7 min instead of maximum 5 min), for another patient (AML4), there was an error of reconstitution volume during product preparation for the second infusion, which lead the patient to receive 108% of the expected dose.

Treatment-related adverse events are shown in table 2. Among the 16 patients treated, one dose-limiting toxicity was observed. The dose-limiting toxicity was CRS grade 4 following the first CYAD-01 infusion in a patient with relapsed or refractory acute myeloid leukaemia (AML15) at dose level three. According to the 3+3 study design, the cohort was expanded to six participants (one extra patient

	Dose level one (n=6)			Dose level two (n=3)			Dose level three (n=7)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Total (any preferred term, highest grade)	4 (67%)	1 (17%)	1 (17%)	3 (100%)	2 (67%)	0	6 (86%)	2 (29%)	3 (43%)
Anaemia	0	0	0	1 (33%)	0	0	0	0	0
Bone pain	1 (17%)	0	0	0	0	0	0	0	0
Bronchial hyperreactivity	0	0	0	0	0	0	1 (14%)	0	0
Chills	1 (17%)	0	0	0	0	0	0	0	0
Cough	0	0	0	0	0	0	1 (14%)	0	0
C-reactive protein increased	0	0	0	0	0	0	1 (14%)	0	0
Cytokine release syndrome*	4 (67%)	0	1 (17%)	3 (100%)	2 (67%)	0	5 (71%)	1 (14%)	1 (14%)
Decreased appetite	1 (17%)	0	0	0	0	0	0	0	0
Diarrhoea	1 (17%)	0	0	0	0	0	0	0	0
Dyspnoea	0	0	0	0	0	0	1 (14%)	0	0
Hypotension	0	0	0	0	0	0	1 (14%)	0	0
Leukocytosis	0	0	0	0	0	0	1 (14%)	0	0
Lymphocyte count decreased	0	1 (17%)	1 (17%)	1 (33%)	0	0	1 (14%)	0	1 (14%)
Neutrophil count decreased	0	0	0	0	0	0	0	1 (14%)	0
Peripheral swelling	1 (17%)	0	0	0	0	0	0	0	0
Thrombocytopenia	0	0	0	0	0	0	0	0	1 (14%)
Increased weight	0	0	0	0	0	0	1 (14%)	0	0
Decreased white blood cell count	0	0	0	0	0	0	0	1 (14%)	0
Patients with at least one adverse event per category									
Cytokine release syndrome*	4 (67%)	0	1 (17%)	3 (100%)	2 (67%)	0	5 (71%)	1 (14%)	1 (14%)
CRES†	0	0	0	0	0	0	0	0	0
Received tocilizumab	0	0	1 (17%)	2 (67%)	2 (67%)	0	3 (43%)	0	1 (14%)
Received corticosteroids	0	0	1 (17%)	0	2 (67%)	0	0	1 (14%)	1 (14%)
Required hospitalisation	1 (17%)	0	1 (17%)	0	1 (33%)	0	0	1 (14%)	1 (14%)
Any dose-limiting toxicity	0	0	0	0	0	0	0	0	1 (14%)
Patients with at least one related serious adverse event	1 (17%)	0	1 (17%)	1 (33%)	2 (67%)	0	0	1 (14%)	1 (14%)

Data are n (%). The group of dose level one had 15 total infusions, dose level two had 12 total infusions, and dose level three had 24 total infusions. *Cytokine release syndrome was graded uniformly according to the CAR-T-cell-therapy-associated TOXicity (CARTOX) Working Group recommendations.[22] †Preferred term encephalopathy or reported term assessed as part of CAR T-cell-related encephalopathy syndrome (CRES); CRES was graded uniformly according to the CARTOX Working Group recommendations.[26] CAR=chimeric antigen receptor.

Table 2: **CYAD-01 treatment-related adverse events per preferred term and per dose level for patients with at least one related adverse event**

was also added to replace a patient who discontinued before day 43). As no other patient in the extended cohort had a dose-limiting toxicity, the maximum tolerated dose was considered as not reached at dose level three. Seven patients had grade 3 or 4 CYAD-01 treatment-related adverse events (table 2), including grade 3 CRS (n=3), grade 4 CRS (n=2, one considered as dose-limiting toxicity), grade 3 or 4 lymphocyte count decrease (n=3), grade 4 thrombocytopenia (n=1), and grade 3 neutrophil and white blood cell count decrease (n=1). No CRES or deaths related to CYAD-01 were reported. CRS was reported for 15 (94%) of 16 patients and was mainly grade 1 or 2 (nine [56%] of 16 patients had grade 1 or 2 CRS after each infusion and 35 [85%] of 41 CRS events were grade 1 or 2). Two patients at dose level two had grade 3 CRS at the third infusion (n=2) or fourth infusion (n=1); one patient at dose level three had grade 3 CRS at the first infusion, and two patients at dose level one and dose level three had grade 4 CRS after the first infusion (and received no further CYAD-01; appendix p 1). Tocilizumab treatment was administered for CRS to six (38%) of all 16 patients, not only for treatment of symptoms but also as prophylactic treatment, especially at subsequent infusions in patients who had already had CRS at one of their first infusions.

Among the 13 patients with relapsed or refractory acute myeloid leukaemia or myelodysplastic syndromes infused with CYAD-01, 12 were evaluable for clinical activity (table 3; figure 2). The patient who had a dose-limiting toxicity upon first CYAD-01 administration (AML15) at dose level three was withdrawn from study treatment and did not proceed to any disease assessment. For all evaluable patients (n=12), the objective response rate and persistence of objective response rate at 3 months were 25% (95% CI 5–57) and 8% (0–38; table 3). Before study enrolment, the responding patient, AML4, (+8/del(7)(q22q36), *FLT3*/*NPM1* wild-type acute myeloid leukaemia) at dose level one had recurrent cytopenias,

bone marrow biopsy confirmed disease relapse with hypocellularity, 6% blasts in numerous clusters, and dysmegakaryopoiesis. At first and second bone marrow evaluations (on days 29 and 57), following treatment with CYAD-01, the patient had a morphological leukaemia-free state, with 2% blasts, normocellularity, and trilineage haematopoiesis, and marked haematological recovery (ie, red blood cell transfusion independence and absolute neutrophil count and platelets) consistent with complete remission with partial haematological recovery.[28] This patient had an allogeneic HSCT on day 98 and was still reported in complete remission on Oct 19, 2022 (ie, for more than 61 months). Another patient at dose level three (AML10), who had 7% bone marrow blasts at baseline, had a complete remission with incomplete haematological recovery at first bone marrow evaluation (day 29; 1·5% bone marrow blasts), which persisted until day 57. This patient received a fourth CYAD-01 administration but did not complete the consolidation cycle as he received another chemotherapy treatment on day 85 and subsequently had allogeneic HSCT on day 157. AML10 withdrew their consent at 5 months and died 30 months after first CYAD-01 infusion. The patient with myelodysplastic syndromes at dose level three (MDS13), who had 9·5% bone marrow blasts at baseline, had a marrow complete remission without haematological improvement at first bone marrow evaluation (day 29; 2% bone marrow blasts) that persisted for 1 month. Four other patients had a best response of stable disease including three lasting at least 3 months. With a median follow-up of 118 days (IQR 46–180), median overall survival was 5·5 months (1·5–8·6; data not shown). Median progression-free survival was 2·8 months (0·9–4·4; data not shown). The best change in bone marrow blast count from baseline in the evaluable patient population and the absolute bone marrow blast changes over time for three responding patients are in the appendix (p 3). One patient (AML8) at dose level two had a relevant bone marrow blast decrease 2 months after the first CYAD-01 infusion and another following initiation of the consolidation cycle and had stable disease for 4·5 months. Two (MM1 and MM2) of the three patients with multiple myeloma enrolled at dose level one were evaluable for clinical activity; neither had a clinical response to CYAD-01 treatment.

Suitable samples for cellular kinetics analysis in peripheral blood were available for ten patients with acute myeloid leukaemia or myelodysplastic syndromes (2 points from dose level one, 3 points from dose level two, and 5 points from dose level three). CYAD-01 transgene concentrations peaked in peripheral blood on average 3·5 days after each infusion (T_{max}), then dropped below the limit of detection within 2 weeks (appendix p 5). The median peak number of transgene copies per µg of DNA in the peripheral blood mononuclear cells (C_{max}) and the area under the curve between day 1 and day 32 ($AUC_{1–32d}$), indicative of CAR T-cell persistence, suggested a dose-dependent effect on cellular kinetics. No cumulative effect was observed with multiple injections. No CYAD-01 cells were detected in the bone marrow of five participants for whom adequate bone marrow aspirate samples were available at month 1 or later timepoints (1 point from dose level one, 3 points from dose level two, and 1 point from dose level 3, none of them corresponding to responder patients).

Serum cytokine and chemokine concentrations obtained before and throughout the treatment administration period were analysed in participants with acute myeloid leukaemia or myelodysplastic syndromes. Before treatment, patients showed heterogeneity in their cytokine and chemokine profiles (measured by Luminex). When grouped by clinical response, all responders showed a pre-treatment Luminex profile similar to that observed in healthy donors (appendix p 6). A cell dose-dependent effect on Luminex score was observed, suggesting that CYAD-01 might modulate cytokine profiles (appendix p 6). Some, but not all, of the CRS events observed were associated with an increase in serum cytokines and chemokines including IL-6, IL-8,

	Dose level one (n=3)	Dose level two (n=3)	Dose level three (n=7)
Complete remission with partial or incomplete haematological recovery or marrow complete remission	1 (33%)	0	2 (29%)
Stable disease	0	2 (67%)	2 (29%)
Progressive disease	2 (67%)	1 (33%)	2 (29%)
Non-evaluable*	0	0	1 (14%)

Data are n (%). *Patient at dose level three with a dose-limiting toxicity upon first CYAD-01 administration was withdrawn from study treatment and did not proceed to any disease assessment.

Table 3: **Best tumour response according to dose level in the acute myeloid leukaemia or myelodysplastic syndrome population**

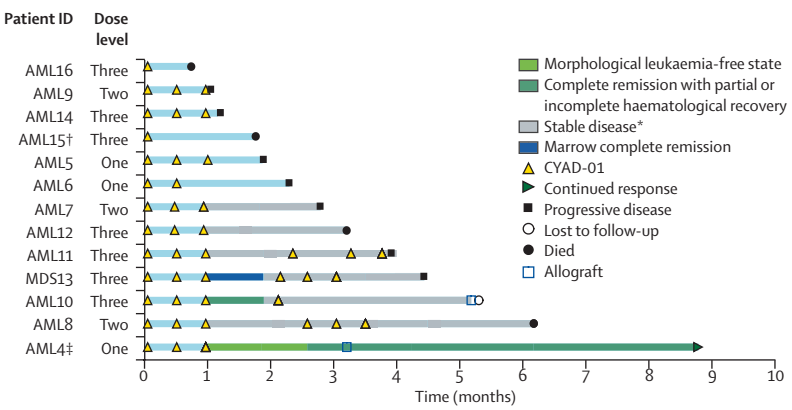

Figure 2: **Time to response and duration of responses in the acute myeloid leukaemia and myelodysplastic syndromes population**

*Lasting at least 3 months. †AML15 had a dose-limiting toxicity upon first infusion and did not receive further CYAD-01 infusions afterwards. ‡AML4 continued in complete remission status after last assessment (the patient was still reported in complete remission at more than 61 months), bar length was shortened to fit graph scale.

IL-1 receptor agonist protein and interferon γ-induced protein-10 (known as IP-10 or CXCL10), but there was no correlation between the CRS grade and the magnitude of cytokine and chemokine alterations.

NKG2D ligand expression at screening was assessed retrospectively in the bone marrow for seven of 13 patients for whom suitable samples were available (two responders, four non-responders, one not evaluable). All samples showed expression of at least two NKG2D ligands (appendix p 6). Compared with other NKG2D ligands, MHC class I chain-related proteins A and B were consistently detected in a high percentage of blasts. The patients who had responded did not show substantially different profiles compared with patients who did not respond (appendix p 6). For three patients, ligand expression post-treatment was analysed at various timepoints. Ligand expression after treatment showed very little or no change, including in the patient with objective response at the time of bone marrow blast reappearance (appendix p 6).

Discussion

To our knowledge, THINK is one of the first phase 1 completed CAR T-cell studies for myeloid malignancies and one of the only studies to use a multiple infusion regimen without preconditioning or bridging chemotherapy. This differentiated strategy is unusual in the current CAR T-cell therapy space but provides the key advantage of understanding the activity of the therapy in the absence of confounding factors, such as the effect of preconditioning on clinical endpoints or safety parameters.

The maximum tolerated dose of CYAD-01 was not reached. The most common grade 3 or worse treatment-related adverse event reported in patients treated with CYAD-01 was CRS, observed in five (31%) of 16 patients, similar to what has been observed for other CAR T-cells in leukaemia.[29] No neurological adverse events (observed in 40–62% of patients with leukaemia treated with CD19-specific CAR T-cells after preconditioning) nor evidence of on-target off-tumour toxicities were observed following infusion of NKG2D CAR T-cells.

Of the 12 evaluable patients, two patients with relapsed or refractory acute myeloid leukaemia and one with relapsed or refractory myelodysplastic syndromes had complete remission with partial haematological recovery, incomplete haematological recovery, or marrow complete remission according to international guidelines.[23,24,28] Two patients with objective responses following CYAD-01 treatment subsequently underwent allogeneic HSCT treatment leading to complete remission each time. Additionally, three patients had stable disease with a durability of at least 3 months, including one patient with 40% marrow blasts decrease. Given the absence of preconditioning or other interventions, the observed clinical activity seems to be directly related to CYAD-01, although there was no obvious correlation between clinical effect and CYAD-01 dosing or peripheral blood engraftment. Our data show CYAD-01 cell dose-dependent cytokine and chemokine modulation, which is indicative of the biological sequelae of CYAD-01 infusion. The study had a small sample size and intrinsically heterogeneous relapsed or refractory acute myeloid leukaemia or myelodysplastic syndromes disease population. Nonetheless, the occurrence of objective responses, although low and non-durable, in these patients with relapsed or refractory acute myeloid leukaemia or myelodysplastic syndromes in the absence of any other confounding treatment is noteworthy.

CYAD-01 showed a short persistence in peripheral blood after each infusion and no CAR T-cells were detected in the bone marrow of patients. These results are expected since the treatment strategy did not include preconditioning chemotherapy, which is believed to support the persistence of infused CAR T-cells through multiple mechanisms, including increase in the homoeostatic cytokines IL-7 and IL-15 and depletion of cytokine sinks.[30,31] Similar C_{max} and T_{max} values were observed after each subsequent infusion, suggesting that anti-CAR immune responses were probably not involved in the clearance of cells. However, expression of NKG2D ligands by CYAD-01 cells after antigen encounter, and subsequent fratricide, could restrict the persistence and functional activity of CYAD-01. To this end, the cellular kinetics of the next generation NKG2D-based CAR T-cell product, CYAD-02, incorporating interfering RNA technology to abrogate the expression of the two main NKG2D ligands (MHC class I chain-related proteins A and B) on the CAR T-cell surface, is being investigated in the phase 1 CYCLE-1 study (NCT04167696). The minimal expansion and persistence of CYAD-01 cells, as compared with CD19-specific CAR T-cells,[32] might also be due to the specificity of the antigen. For B-cell haematological malignancies, in contrast to acute myeloid leukaemia and myelodysplastic syndromes, CAR T-cells encounter their targets on the host's normal and malignant B-cells, which act as antigen-presenting cells thereby providing strong proliferation signals and promoting CAR T-cell persistence. In addition, the paucity of administration of preconditioning chemotherapy would lead to a rapid decline of the frequency of CAR T-cells due to the absence of proliferation signals (including IL-7 and IL-15). In this context, the addition of cyclophosphamide and fludarabine as a preconditioning regimen before CYAD-01 infusion is evaluated in the phase 1 DEPLETHINK study (NCT03466320) in a similar patient population.

Clinical-grade manufacturing remains a crucial aspect of all cell therapies, especially for those derived from autologous material. The incidence of increased manufacturing failures with peripheral blood blasts above 30% at baseline does have the potential to slow the development of CAR T-cells for myeloid indications.

In conclusion, CYAD-01 is an autologous CAR T-cell therapy that has shown good tolerability but low activity in patients with relapsed or refractory acute myeloid leukaemia and myelodysplastic syndromes, with additional optimisation needed. The results presented here support further development, including the use of preconditioning therapy, and further engineering of NKG2D-based CAR products.

Contributors

DAS, TK, CL FFL, and AF and were involved in the study conception and design, manuscript writing, and approval. DAS, TK, VH, XP, PL, ESW, JBB, IM, J-PM, and AA enrolled and treated patients, and gathered data. EMA-O, RB, NB, M-SD, and AF analysed and interpreted data. MLD and DEG reviewed data and provided scientific input. All authors participated in writing the Article, provided feedback throughout the development process, and approved the final submitted version. EMA-O, RB, NB, M-SD, and AF had access and verified the data. All authors had full access to all the data in the study and had final responsibility for the decision to submit publication.

Declaration of interests

DAS has received payment as a member of advisory boards for Aprea, AvenCell, BlueBird Bio, Bristol Myers Squibb (BMS), Intellia, Kite, Novartis, Shattuck Labs, Servier, and Syndax; as a consultant for AbbVie, Magenta, Molecular Partners, and Takeda; as a member of a speakers bureau for BMS, Incyte, and Servier; and for research funding from Aprea and Jazz. VH has received payment as speaker with honoraria from Novartis, support for attending meetings from Novartis and Abbvie, and as member of data safety monitoring board with Incyte and Novartis. ESW has received consulting fees from Abbvie, AmGen, Astellas, BMS, Gilead, GlaxoSmithKline, Janssen, Jazz, Kite, Mana, Novartis, NuProbe, Pfizer, PharmaEssentia, and Takeda; and payment as member of data safety monitoring board or speaker bureau from Abbvie, Astellas, CTI Biopharma, Dava oncology, Gilead, Novartis, Kite, Pfizer, Rafael, and Stemline. MLD has received licensing fees from Atara and CRISPR; research funds from Atara, CRISPR, Kite, and Novartis; and consulting fees from Synthekine, Adicet, and Bellicum. J-PM has received payment as an advisory board member or speaker with honoraria from Pfizer, Roche, AstraZeneca, Bayer, Innate, Merck Serono, Boerhinger, BMS, Novartis, Janssen, Incyte, Cue Biopharma, ALX Oncology, iTEOS, eTheRNA, NEKTAR, and F-Star; and as member of a data safety monitoring board with honoraria for Psioxus. AA took part in advisory boards from Amgen, AstraZeneca, Bayer, Daiichi, EISAI, Genomic Health, Hengrui, Innate, Ipsen, Leo Pharma, Lilly, Merck, MSD, Novartis, Pfizer, and Seattle Genetics; received speaker fees from Amgen, AstraZeneca, Bayer, Daiichi, EISAI, Genomic Health, Ipsen, Leo Pharma, Lilly, Merck, Merck Sharpe and Dohme, Novartis, Pfizer, and Seattle Genetics; and research grants from BMS, Roche. EMA-O, RB, NB, M-SD, DEG, CL, FFL and AF are or were employed by Celyad Oncology. RB, NB, M-SD, DEG, CL, FFL, and AF are or were employed by Celyad Oncology. All other authors declare no competing interests.

Data sharing

Upon email request (contactus@celyad.com), individual participant de-identified datasets will be made available on a case-by-case basis. Supporting documents that will be available upon request include the study protocol, the informed consent form, and statistical analysis plan.

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