Poster 3032

CYAD-101: An innovative non-gene edited allogeneic CAR-T for solid tumor cancer therapy

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BACKGROUND

- **Allogeneic chimeric antigen receptor (CAR) T-cells** are produced from healthy donor cells, can be banked and used in a timely manner, avoiding the time delay associated with the manufacture of an autologous cell product.
- While allogeneic CAR T-cell approaches have started to show some traction in hematological cancers in humans, to date, researchers have struggled to find similar success in more challenging solid tumor indications.
- **CYAD-101** is a **novel, first-in-class, allogeneic** NKG2D receptor-based **CAR T-cell therapy** (Figure 1).

Figure 1: CYAD-101 construct

- The allogeneic CYAD-101 CAR T-cell product uses a **non-gene edited** T-cell receptor (TCR) inhibitory molecule (TIM) to control **graft-versus-host disease** (GvHD), combined with a CAR based on the natural killer group 2D (NKG2D) receptor.
  - The NKG2D CAR comprises the full-length human NKG2D receptor fused to the intracellular domain of CD3ζ.
  - NKG2D binds eight stress ligands that are over-expressed in a broad range of solid tumors including **metastatic colorectal cancer** (mCRC).
  - TIM is a truncated peptide that competes with the endogenous CD3ζ in the TCR complex formation, hence reducing the ability of the endogenous TCR to drive T-cell activation, while not impacting the activity of the NKG2D CAR.
- There are few treatment options available for mCRC patients who progress after the second line therapy. mCRC patients with ≥ 4 lines of therapy have a median progression-free survival of 1.7-3.2 months with best supportive care [1].
- Clinical grade CYAD-101 cells were produced for the **Phase 1 alloSHRINK study** (NCT03692429) which evaluated the concurrent administration of CYAD-101 with FOLFOX (Folinic acid [leucovorin], fluorouracil [5-FU] and oxaliplatin) chemotherapy.
- The use of FOLFOX was primarily as a lymphodepletion regimen to facilitate CYAD-101 engraftment and avoid **host-versus graft** (HvG) reaction while also inducing an up-regulation of NKG2D ligand expression on the cancer cells.

  FOLFOX was not intended to induce a cytotoxic effect on the cancer cells as most patients were refractory to previous treatment lines.

[1] Sonbol et al. 2019. Oncologist. 24(9):1174-1179.

METHODS

- A bank of > 53 billion clinical grade CYAD-101 cells was generated in two production runs using T-cells from one healthy donor apheresis. This was sufficient for dosing all patients in both the dose escalation segment and the small expansion segment of this Phase I alloSHRINK study (15 patients in total) with 3 injections per patient, as planned.
- The CYAD-101 product generated with both production runs was highly consistent and composed of 85% CD4+ T-cells with a transduction level of > 92%, low relative expression of CD69/CD25 and no detectable expression of PD-1/LAG-3.
- The CYAD-101 cells were predominantly (>80%) cluster of differentiation (CD)45RA-/CD27- suggestive of an effector memory T-cell population.
- The **Phase I alloSHRINK study**:
  - **Patient population**
    Non-resectable mCRC patients with recurrent/ progressing disease after at least one metastatic treatment line including oxaliplatin and irinotecan-based chemotherapy.
  - **Primary objective**
    Definition of the recommended dose based on the occurrence of dose-limiting toxicity (DLT) during the treatment phase.
  - **Study design**
    Dose escalation segment with a Fibonacci 3+3 study design and 3 dose levels (DLs) of CYAD-101 ($1x10^8$, $3x10^8$ and $1x10^9$ cells per injection), and small expansion segment with 6 additional patients at the recommended dose.
  - **Treatment schedule**
    Three intravenous CYAD-101 injections at a 2-week interval and at Day 3 of three consecutive FOLFOX chemotherapy cycles (standard doses).

TABLES & FIGURES

Table 1: Patient, tumor and treatment characteristics

	TOTAL N=15
Mean age and range (year)	56 (25-74)
Male / Female	10 / 5
ECOG Grade 0 / 1	6 / 9
Site of primary tumor	
Right / left colon / rectum	2 /8 /5
Sites of target lesions [a]	
Liver	8 (57.1%)
Lung	5 (35.7%)
Peritoneum	3 (21.4%)
Retroperitoneum	3 (21.4%)
Other	2 (14.3%)
Microsatellite stable	15 (100%)
RAS mutant	10 (66.7%)
BRAF mutant [b]	2 (15.4%)
Number of target lesions [a]	
1	3 (21.4%)
2 to 3	10 (71.4%)
4	1 (7.1%)
Prior adjuvant treatment	4 (26.7%)
Number of prior metastatic treatment lines	
1	3 (20%)
2 to 3	6 (40%)
≥ 4	6 (40%)
Mean (range)	3 (1-6)
Number of prior metastatic lines containing oxaliplatin	
0 [c]	1 (6.7%)
1	10 (66.7%)
2	4 (26.7%)
Number of patients with prior metastatic treatment lines containing molecular-targeting agents	
EGFR targeted therapy	6 (40%)
Bevacizumab	11 (73.3%)

[a] At the data lock point (uncleaned database), 1 patient with progressive disease had target lesions at unknown site(s).
[b] Two patients had unknown BRAF mutation status.
[c] The patient developed metastases during adjuvant FOLFOX treatment.
EGFR = epidermal growth factor receptor

Table 2: Incidence of adverse events (AEs)

	TOTAL N=15 (44 injections) All AEs	TOTAL N=15 (44 injections) All treatment-related AEs
Any Grade	183 (100%)	18 (100%)
Grade ≥ 2	68 (37.2%)	3 (16.7%)
Grade ≥ 3	19 (10.4%)	0 (0%)
Grade ≥ 4	3 (1,6%)	0 (0%)
Dose limiting toxicity	0 (0%)	0 (0%)
Serious adverse events	4 (2.2%)	1 (5.6%) [a]
AEs leading to treatment discontinuation	0 (0%)	0 (0%)

[a] Cytokine Release Syndrome Grade 1
AEs = adverse events; N = number of patients. Data from uncleaned database.

Table 3: Treatment-related AEs

	TOTAL N=15 (44 injections) All	Grade ≥ 3
Patients with any related AE (%)	7 (46.7%)	0 (0%)
Arthralgia	2 (13.3%)	0 (0%)
Diarrhea	2 (13.3%)	0 (0%)
Cytokine Release Syndrome	2 (13.3%)	0 (0%)
Headache	1 (6.7%)	0 (0%)
Myalgia	1 (6.7%)	0 (0%)
Chills	1 (6.7%)	0 (0%)
Feeling hot	1 (6.7%)	0 (0%)
Hypertension	1 (6.7%)	0 (0%)
Abdominal pain	1 (6.7%)	0 (0%)
Decreased appetite	1 (6.7%)	0 (0%)
Hyperesthesia	1 (6.7%)	0 (0%)
Erythema	1 (6.7%)	0 (0%)
Injection site coldness	1 (6.7%)	0 (0%)
Musculoskeletal stiffness	1 (6.7%)	0 (0%)
C-reactive protein increased	1 (6.7%)	0 (0%)

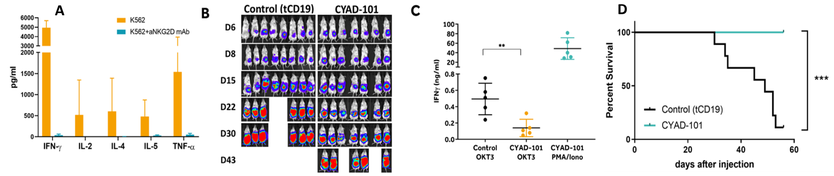
N=number of patients. Data from uncleaned database.

Table 4: Best tumor responses (RECIST 1.1)

Clinical outcome	TOTAL N=15 (44 injections)
Best overall responses – n (%)	
Partial response (PR)	2 (13%)
Stable disease (SD)	9 (60%)
Progressive disease (PD)	4 (27%)
Objective response rate [a] - %	13%
Disease control rate [b] – %	73%

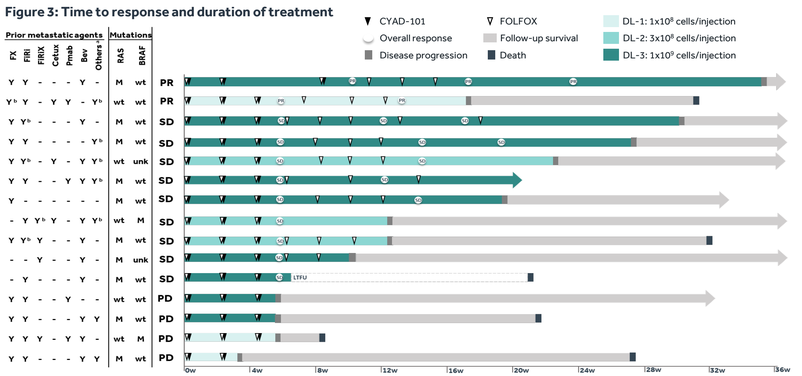
N=number of patients. Data from uncleaned database.

Figure 2: Preclinical validation of the CYAD-101 clinical runs



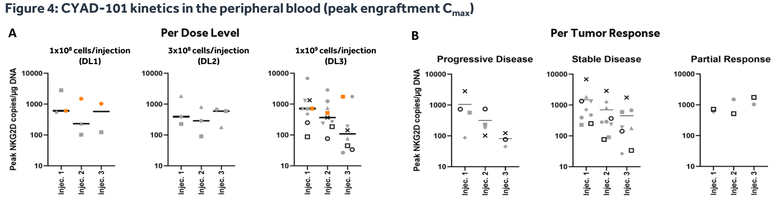
(A) Cytokine production by CYAD-101 upon co-culture with K562 cancer cells in the presence or absence of αNKG2D blocking antibody. **(B)** In vivo anti-tumor activity of CYAD-101 against an established orthotopic HCT-116 CRC tumor model. **(C)** IFN-g production by CYAD-101 upon activation by αCD3 (clone OKT3) or phorbol myristate acetate/Ionomycin. **(D)** CYAD-101 failed to induce significant levels of GvHD upon intravenous injection in irradiated NSG mice, in contrast to control T-cells from the same donor. $^{**}p < 0{,}01$, $^{***}p < 0{,}001$

Figure 3: Time to response and duration of treatment



[a] Other metastatic treatment lines: Regorafenib, trifluridine/tipiracil, undisclosed Phase I/II agent, checkpoint inhibitor, aflibercept, binimetinib, encorafenib, liver embolization, liver selective internal radiotherapy with Yttrium 90 spheres
[b] ≥ 2 metastatic treatment lines
Bev = Bevacizumab; Cetux = Cetuximab; FX = FOLFOX; FiRi = FOLFIRI; FiRiX = FOLFIRINOX; LTFU: lost to follow-up; M = mutation; Pmab = Panitumumab; PD = progressive disease; PR = partial response; SD = stable disease; unk = unknown; w = week; wt = wild type. Data from uncleaned database.

Figure 4: CYAD-101 kinetics in the peripheral blood (peak engraftment C_{max})



C_{max} = maximum concentration

Table 5: HLA typing of donor and patients demonstrates minimal HLA matching and no correlation with clinical activity

DL	Best Resp.	HLA locus	A		B		C		DRB1		DQB1		DPB1		Mismatches		
		allele	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	#1	#2	All alleles	Class I	Class II
		CYAD-101	02:01	02:01	51:01	39:01	14:02	12:03	15:01	08:01	06:02	04:02	02:01	23:01			
DL1	PR	**Pt 01**	03:01	24:02	15:01	39:01	03:04	12:03	07:01	13:01	03:03	06:03	04:02	04:02	10	4	6
DL1	PD	**Pt 02**	02:01	02:01	15:01	35:01	03:04	04:01	01:01	04:01	03:02	05:01	04:01	04:02	10	4	6
DL1	PD	**Pt 03**	01:01	02:01	08:01	51:01	07:01	15:02	03:01	15:01	02:01	05:02	01:01	04:02	9	4	5
DL2	SD	**Pt 04**	02:01	11:01	14:01	40:01	03:04	08:02	04:01	07:01	02:01	03:01	02:01	03:01	10	5	5
DL2	SD	**Pt 05**	01:01	68:01	08:01	18:01	07:01	07:01	03:01	11:04	02:01	03:01	01:01	04:02	12	6	6
DL2	SD	**Pt 06**	24:02	24:02	07:02	07:02	07:02	07:02	01:01	15:01	05:01	06:02	04:01	17:01	10	6	4
DL3	SD	**Pt 07**	01:01	24:02	08:01	35:01	03:03	07:01	03:01	04:04	02:01	03:02	11:01	112:01*	12	6	6
DL3	PR	**Pt 08**	03:01	30:01	13:02	40:01	03:04	06:02	04:01	13:02	03:01	06:04	02:01	04:01	11	6	5
DL3	PD	**Pt 09**	03:01	31:01	18:01	35:01	04:01	07:01	11:01	11:01	03:01	03:01	04:01	04:01	12	6	6
DL3	SD	**Pt 10**	02:01	03:01	49:01	51:01	02:02	07:01	04:05	11:01	03:02	03:01	02:01	13:01	9	4	5
DL3	SD	**Pt 11**	11:01	24:02	37:01	52:01	06:02	12:02	10:01	11:01	03:01	05:01	04:01	04:01	12	6	6
DL3	SD	**Pt 12**	02:03	11:01	15:02	57:01	06:02	08:01	07:01	12:02	02:01	03:01	02:02	04:01	12	6	6
DL3	SD	**Pt 13**	30:02	68:02	18:01	53:01	04:01	05:01	03:01	13:02	02:01	06:04	04:01	05:01	12	6	6
DL3	PD	**Pt 14**	03:01	26:01	07:02	38:01	07:02	12:03	01:01	16:01	05:01	05:02	02:01	04:02	10	5	5
DL3	SD	**Pt 15**	11:01	31:01	35:01	51:01	04:01	14:02	03:01	14:01	02:01	05:03	02:01	03:01	9	4	5

DL = dose level; HLA; human leucocyte antigen; PD = progressive disease; PR = partial response;
pt = patient; Resp. = response; SD = stable disease

Green boxes indicate HLA matching between donor and patient (uncleaned database).
* = rare allele

MAIN RESULTS

- Upon co-culture with target K562 cells, CYAD-101 readily produced IFN-γ that was blocked by a NKG2D blocking antibody confirming specificity of the CAR. CYAD-101 cells produced *in vitro* an array of Th1 (IFN-γ, IL-2 and TNF-α) and Th2 (IL-4, IL-5) cytokines (Figure 2A). Importantly, minimal IFN-γ was produced upon TCR stimulation while stimulation with a non-TCR mitogen (PMA + ionomycin) lead to high levels of IFN-γ (Figure 2C). Data also confirmed that CYAD-101 maintained CAR-directed anti-tumor activity (Figure 2B) in the absence of the induction of GvHD (Figure 2D).
- 15 mCRC patients have been treated in this Phase I alloSHRINK study (3 at DL 1, 3 at DL2 and 9 at DL3) (Table 1).
- 7 out of 15 patients reported treatment-related adverse events. All were of Grade 1 or Grade 2 (Table 3). Importantly, no sign of clinical or biological GvHD have been observed.
- **Best overall response**: Out of 15 patients, 2 patients with PR confirmed by RECIST 1.1. criteria and 9 patients with SD, including 7 SD ≥ 3 months, with 1 ongoing (Table 4 and Figure 3).
- No correlation was observed between clinical responses and the degree of human leucocyte antigen (HLA) mismatch between CYAD-101 cells and patients (Table 5).
- CYAD-101 cells were detected in the peripheral blood by polymerase chain reaction-based methods. No dose-dependent effect was observed. However, a drop in C_{max} at DL3 after the 2nd and 3rd injections was observed for 10 out of 15 patients (Figure 4A). Interestingly, the two patients with PR did not exhibit this C_{max} drop (Figure 4B).

CONCLUSIONS

- CYAD-101 is a novel, first-in-class, allogeneic NKG2D receptor-based CAR T-cell therapy.
- To our knowledge, the alloSHRINK study (NCT03692429) is the **first clinical study** that used **a non-gene edited allogeneic CAR T-cell therapy in a solid cancer indication.**
- CYAD-101 demonstrated a **favorable safety profile** at all evaluated dose-levels, with no DLT nor GvHD observed.
- The dose of $1x10^9$ CYAD-101 cells per injection post-FOLFOX chemotherapy is defined as the recommended dose for further development.
- The response rate of **two patients with confirmed PR**, including one KRAS mutated patient, out of 15 patients is an **encouraging indicator of clinical activity in mCRC patients** with recurrent/progressing disease after at least one metastatic treatment line including oxaliplatin and chemotherapy. No evident correlation could be observed between disease control and patient baseline characteristics.
- The drop in C_{max} observed after the 2nd and 3rd CYAD-101 injections is possibly the result of a more rapid clearance of CAR T-cells. Despite use of FOLFOX as a lymphodepletion regimen, the development of a host versus graft reaction cannot be ruled out and warrants further investigations.
- Overall safety and clinical activity data are HLA-independent indicating that **CYAD-101 cells can be used in a broad population regardless of the HLA haplotype**.
- These early clinical results demonstrating the safety of a non-gene edited allogeneic CAR T-cell approach with no signs of graft versus host disease together with the clinical activity observed in incurable mCRC patients **support the further development of the CYAD-101 allogeneic CAR T-cells**.

ACKNOWLEDGMENTS & DISCLOSURES

- Celyad thanks patients & their families, physicians, and study teams at all participating centers.
- CYAD-101 clinical batches have been manufactured at Cellular Therapeutics Ltd's (CTL), Manchester, UK.
- This study was funded and sponsored by Celyad SA (ClinicalTrials.gov identifier: NCT03692429).
- HP, JD, SA, AH and EVC are Investigators of the alloSHRINK study. AM, PAS, DEG, SM, SS, EC, CL, AF and FFL are employed by Celyad SA.

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