Phase 1 studies assessing the safety and clinical activity of autologous and allogeneic NKG2D-based CAR T therapy in metastatic colorectal cancer

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INTRODUCTION

- **Chimeric antigen receptor T-cell** (CAR T-cell) therapies have achieved great efficacy in some very specific types of hematological malignancies. However, they have thus far failed to deliver significant objective responses in solid tumors likely due to the immune suppressive tumor microenvironment (TME) that renders infiltration and traffic of CAR T-cells extremely difficult.
- Current CAR T-cell therapies rely mostly on the patient's own cells (autologous approach) associated with a high variability in the starting material and time pressure for manufacturing. The use of healthy donor cells (allogeneic approach) can circumvent these issues.
  Allogeneic reactivity is the primary concern for allogeneic CAR T-cell therapies due to: (i) host-versus-graft reaction (HvGR), and (ii) graft-versus-host disease (GvHD). The HvGR can be transiently controlled by chemotherapy enabling engraftment of the allogeneic CAR T cells.
- **NKG2D CAR T-cells** are CAR T-cells based on the natural full-length human natural killer group 2D (NKG2D) receptor fused to the intracellular domain of CD3ζ, the former targeting 8 stress ligands whose expression is found in many types of solid cancers including colorectal cancer.
- Celyad is currently pursuing the clinical development of two NKG2D CAR T-cell product candidates:
  - **CYAD-01**, *autologous* NKG2D CAR T-cells has been tested as a monotherapy without pre-conditioning chemotherapy and showed initial clinical responses including transient complete remissions in relapsed/refractory acute myeloid leukemia patients and transient stable disease in metastatic colorectal cancer (mCRC) patients (THINK study – NCT03018405) [1,2];
  - **CYAD-101**, *allogeneic* counterpart of CYAD-01 using a non-gene editing technology (T-cell receptor [TCR] inhibitory molecule, [TIM]) to prevent GvHD through inhibition of the TCR signal.

1. Sallman DA et al. (2018), Haematologica 103: e242-e246.
2. Presented at the Society for Immunotherapy of Cancer (SITC) Congress (November 2018) (P255).

CLINICAL DEVELOPMENT IN METASTATIC CRC (mCRC)

- Two Phase I studies with a similar design are currently evaluating the safety and the engraftment of the two NKG2D CAR-T cell product candidates in mCRC patients:
  - **SHRINK study** (NCT03310008) evaluates the **autologous CAR T-cell product CYAD-01**;
  - **ALLOSHRINK study** (NCT03692429) evaluates the **allogeneic CAR T-cell product CYAD-101**.
    To our knowledge, the ALLOSHRINK study is the first study exploring the safety and tolerability of a non-gene edited, allogeneic CAR T cell therapy in a solid cancer.
- The studies are conducted in the following mCRC patient populations (see also demographics below):
  - The SHRINK study is conducted in two different mCRC patient populations: (i) resectable liver dominant mCRC with FOLFOX chemotherapy as 1st line treatment (ie, **neoadjuvant** population), and (ii) non-resectable mCRC with prior chemotherapy lines for mCRC including FOLFOX and/or FOLFIRI (ie, **rechallenge** population);
  - The ALLOSHRINK study is only conducted in the rechallenge population.
- **SHRINK and ALLOSHRINK share the same study design:**
  - Dose escalation with a Fibonacci 3+3 design;
  - Three dose levels (DL) of the NKG2D CAR T-cells: $1x10^8$, $3x10^8$ and $1x10^9$ cells per infusion;
    Note: recruitment in the ALLOSHRINK study at DL-3 is still ongoing and not reported.
  - Multiple intravenous CAR T-cell infusions at a 2-week interval administered at Day 3 of concurrent FOLFOX chemotherapy cycles.
    FOLFOX is known to (i) induce NKG2D ligand expression on CRC tumor cells, and (ii) induce a transient lymphodepletion that can facilitate CAR T engraftment. Together, these characteristics should enhance the activity of CYAD-01/CYAD-101 that could be combined with chemotherapy to produce greater clinical response rates.

RESULTS

DEMOGRAPHICS	SHRINK STUDY (AUTOLOGOUS CYAD-01) DL-1 (1×10^8) N=3	DL-2 (3×10^8) N=3	DL-3 (1×10^9) N=3	TOTAL N = 9	ALLOSHRINK STUDY (ALLOGENEIC CYAD-101) DL-1 (1×10^8) N=3	DL-2 (3×10^8) N=3	TOTAL N = 6
Age (years): Mean (range)	55 (53-57)	49 (28-63)	64 (59-73)	**56 (28-73)**	60 (46-74)	59 (54-62)	**59,5 (46-74)**
Gender: Male/Female	0/ 3	2 / 1	2 / 1	**4 / 5**	2 / 1	2 / 1	**4 / 2**
ECOG: Grade 0 / 1	3 / 0	1 / 2	2 / 1	**6 / 3**	0/ 3	0/ 3	**0/ 6**
LVEF (%): Mean (range)	67 (65-70)	68 (65-71)	57 (55-61)	**64 (55-71)**	60 (51-74)	60 (58-62)	**60 (51-74)**
Nb. of patients with neoadjuvant treatment	3	0	1	**4**	Not applicable	Not applicable	**Not applicable**
Nb. of patients with prior treatment lines for mCRC	0	3	2	**5**	3	3	**6**
Nb. of prior metastatic treatment lines: Mean (range)	Not applicable	3 (1-5)	4 (3-5) *	**3 (1-5)***	4 (3-5)	5 (4-5)	**4 (3-5)**
Nb. of patients with ≥ 1 prior FOLFOX / FOLFIRI (rechallenge)	0	3	2	**5**	3	3	**6**

*For rechallenge population only

RELATED ADVERSE EVENTS (AE)	SHRINK STUDY (AUTOLOGOUS CYAD-01) DL-1 (1×10^8) 9 infusions over 3 patients				DL-2 (3×10^8) 15 infusions over 3 patients				DL-3 (1×10^9) 12 infusions over 3 patients				TOTAL 36 infusions over 9 patients			
	Grade				Grade				Grade				Grade			
	All	1	2	3	All	1	2	3	All	1	2	3	All	1	2	3
AE Preferred Term																
Total patients with ≥ 1 related AE (%)	2	1	-	1	2	2	-	-	2	2	1	-	**6 (66.7)**	**5 (55.6)**	**1 (11.1)**	**1 (11.1)**
Cytokine release syndrome (CRS)	-	-	-	-	1	1	-	-	1	1	-	-	**2 (22.2)**	**2 (22.2)**	**-**	**-**
Infusion site reaction	1	1	-	-	-	-	-	-	-	-	-	-	**1 (11.1)**	**1 (11.1)**	**-**	**-**
Pyrexia	-	-	-	-	1	1	-	-	2	2	-	-	**3 (33.3)**	**3 (33.3)**	**-**	**-**
INR increase	-	-	-	-	-	-	-	-	1	-	1	-	**1 (11.1)**	**-**	**1 (11.1)**	**-**
Anemia	1	-	-	1	-	-	-	-	-	-	-	-	**1 (11.1)**	**-**	**-**	**1 (11.1)**
Fatigue	-	-	-	-	1	1	-	-	-	-	-	-	**1 (11.1)**	**1 (11.1)**	**-**	**-**
Atrial tachycardia	-	-	-	-	1	1	-	-	-	-	-	-	**1 (11.1)**	**1 (11.1)**	**-**	**-**
Abdominal pain	-	-	-	-	-	-	-	-	-	-	-	-	**-**	**-**	**-**	**-**
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-	**-**	**-**	**-**	**-**
Decreased appetite	-	-	-	-	-	-	-	-	-	-	-	-	**-**	**-**	**-**	**-**

INR: International Normalized Ratio

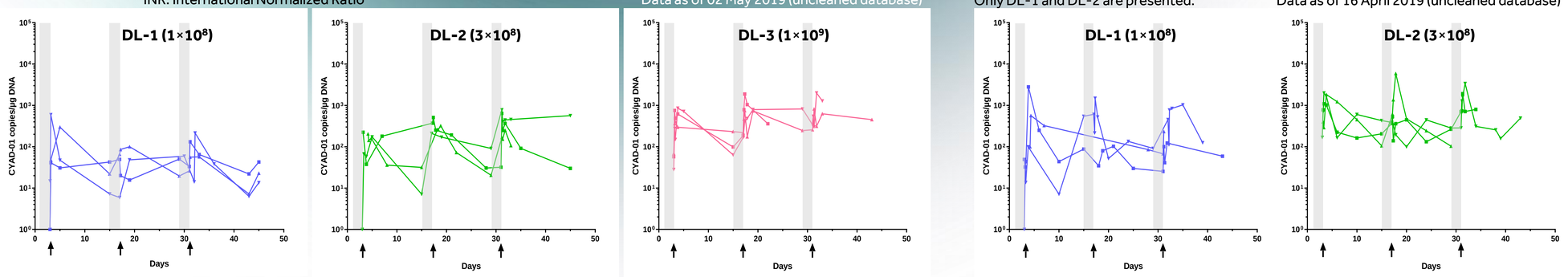
Data as of 02 May 2019 (uncleaned database)

	ALLOSHRINK STUDY (ALLOGENEIC CYAD-101) DL-1 (1×10^8)				DL-2 (3×10^8)				TOTAL			
	8 infusions over 3 patients				9 infusions over 3 patients				17 infusions over 6 patients			
	Grade				Grade				Grade			
	All	1	2	3	All	1	2	3	All	1	2	3
AE Preferred Term												
Total patients with ≥ 1 related AE (%)	-	-	-	-	1	1	-	-	**1 (16.7)**	**1 (16.7)**	**-**	**-**
Cytokine release syndrome (CRS)	-	-	-	-	-	-	-	-	**-**	**-**	**-**	**-**
Infusion site reaction	-	-	-	-	-	-	-	-	**-**	**-**	**-**	**-**
Pyrexia	-	-	-	-	-	-	-	-	**-**	**-**	**-**	**-**
INR increase	-	-	-	-	-	-	-	-	**-**	**-**	**-**	**-**
Anemia	-	-	-	-	-	-	-	-	**-**	**-**	**-**	**-**
Fatigue	-	-	-	-	-	-	-	-	**-**	**-**	**-**	**-**
Atrial tachycardia	-	-	-	-	-	-	-	-	**-**	**-**	**-**	**-**
Abdominal pain	-	-	-	-	1	1	-	-	**1 (16.7)**	**1 (16.7)**	**-**	**-**
Diarrhea	-	-	-	-	1	1	-	-	**1 (16.7)**	**1 (16.7)**	**-**	**-**
Decreased appetite	-	-	-	-	1	1	-	-	**1 (16.7)**	**1 (16.7)**	**-**	**-**

Only DL-1 and DL-2 are presented.

Data as of 16 April 2019 (uncleaned database)

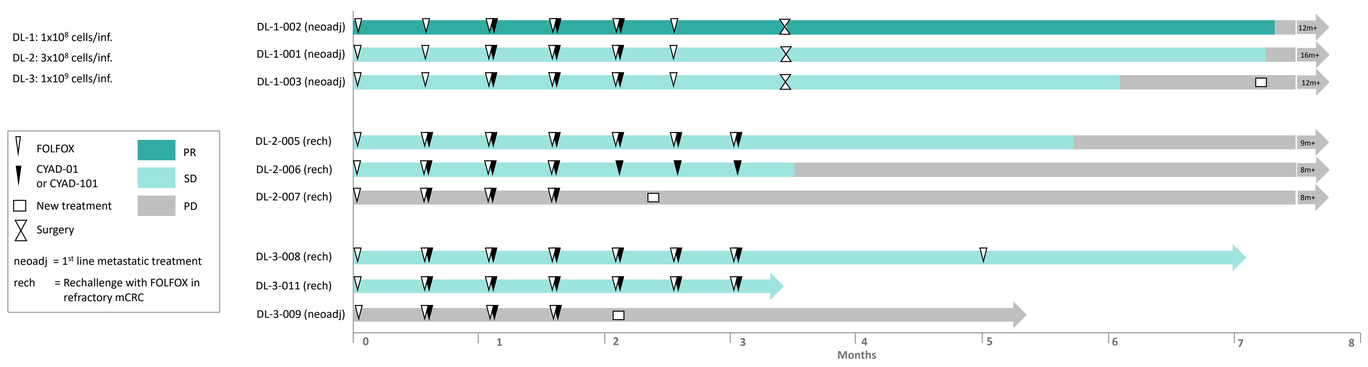
CAR T-CELL PHARMACOKINETICS (in the peripheral blood)



SHRINK (autologous CYAD-01) ↑ days of CYAD-01 infusion FOLFOX chemotherapy

ALLOSHRINK (allogeneic CYAD-101) ↑ days of CYAD-101 infusion FOLFOX chemotherapy

BEST OVERALL RESPONSE	SHRINK STUDY (AUTOLOGOUS CYAD-01) DL-1: 1×10^8 cells/infusion (3 patients)	DL-2: 3×10^8 cells/infusion (3 patients)	DL-3: 1×10^9 cells/infusion (3 patients)	ALLOSHRINK STUDY (ALLOGENEIC CYAD-101) DL-1: 1×10^8 cells/infusion (3 patients)	DL-2: 3×10^8 cells/infusion (3 patients)
Complete response (CR)					
Partial response (PR)	1/ 3 (neoadjuvant)			1/ 3	
Stable disease (SD) (≥ 3 months)	2/ 3 (neoadjuvant)	2/ 3	2/ 3		3/ 3
Progressive disease (PD)	1/ 3	1/ 3 (neoadjuvant)		2/ 3	



CONCLUSIONS

- **No** clinical evidence of **GvHD** has been recorded in the ALLOSHRINK study evaluating the allogeneic product candidate CYAD-101.
- **The host versus graft response against the allogeneic CYAD-101** cells appears to be controlled as evidenced by the similar levels of CYAD-101 engraftment after 2nd and 3rd infusions.
- Both autologous CYAD-01 and allogeneic CYAD-101 concurrently administered with FOLFOX demonstrated a **favorable safety profile** with no reports of CRS > Grade 2, related serious adverse events, dose-limiting toxicities, nor on-target off-tumor toxicity.
- **Initial observations of partial responses and stable disease with CYAD-01** (best overall response ≥ 3 months 1 PR, 6 SD of 9 total patients) **and with CYAD-101** (1PR, 3 SD of 6 total patients) provides encouragement for the combination of this CAR T approach with FOLFOX in difficult-to-treat solid cancer mCRC.
- Preliminary data show a **dose-dependent effect on the cell kinetics** with higher levels of engraftment with increasing doses of CAR T-cells for both the autologous CYAD-01 and allogeneic CYAD-101 product candidates.
- Noteworthy, the allogeneic CYAD-101 product appears to provide increased levels of relative engraftment (time-averaged/area under the curve) compared to the autologous CYAD-01 product at the same doses.
- To our knowledge, the ALLOSHRINK study is the **first study with an allogeneic CAR T-cell therapy in a solid cancer indication** and our preliminary data show no evidence of GvHD.