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Brief communication

Celyad's novel CAR T-cell therapy for solid malignancies

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ABSTRACT

Celyad recently initiated several clinical trials with the CYAD-01 product, a natural killer group 2D (NKG2D)-based chimeric antigen receptor (CAR), in both solid and hematologic tumor types. This review discusses the unique properties of CYAD-01, expecting to provide a new paradigm to fight against solid tumors.

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Chimeric antigen receptor (CAR) T-cell (CAR-T) therapy has become one of the most innovative and successful immunotherapy of cancer in recent times. However, this success has been restricted only to specific hematologic malignancies (acute lymphoblastic leukemia and non-Hodgkin's lymphoma) and that too restricted to only specific indications.

The CAR is a genetically engineered construct which provides T-cells a pre-determined specificity [1]. These constructs have been developed through several generations and are generally based on the same configuration: an extracellular antigen binding domain (generally a single-chain variable fragment (scFv) derived from an antibody) linked through an extracellular spacer domain to a transmembrane domain and, finally, one or several intracellular signaling domains. To date, the most investigated target for CAR T-cell therapy has been CD19, an antigen expressed by most B-cell malignancies, but not in normal tissues other than those originating from the B-cell lineage. CD19-specific CAR T-cell therapy has demonstrated impressive clinical responses in patients with advanced, chemotherapy-resistant leukemia and lymphoma, reaching up to 70 to 90% of minimum residual

disease-negative complete remissions in some studies [2–4]. Consequently, two CD19-specific CAR-T treatments were recently approved by the U.S. Food and Drug Administration (FDA), *i.e.* Yescarta™ from Kite Pharma/Gilead (a.k.a. axicabtagene ciloleucel) and Kymriah™ from Novartis Pharmaceuticals (a.k.a. tisa-genelecleucel), for patients with relapsed or refractory aggressive non-Hodgkin lymphoma (NHL) or acute lymphoblastic leukemia (ALL), respectively.

The successes observed with CAR-T therapies in hematological malignancies has however yet to be observed within solid tumor malignancies, mainly because of numerous challenges specifically posed by solid tumors [5–8]. In brief, these include the lack of suitable tumor specific target antigens that can be exploited with no inherent risk of on-target off-tissue toxicity, the relative heterogeneity within the tumor, the need to control the barriers that prevent lymphocyte trafficking into the tumor microenvironment (TME) and tumor stroma, and finally, the need to overcome a hostile immunosuppressive tumor microenvironment, which is generally responsible of CAR-T exhaustion. This combination of hurdles has not yet been overcome using classical antibody-based

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CAR technology since most previous attempts at investigating this technology among solid cancer indications have been proven unsuccessful [9].

Celyad has developed a CAR construct based on the full-length natural killer group 2D (NKG2D) receptor fused with the CD3 ζ signaling domain, called CYAD-01 (a.k.a. NKR-2). NKG2D is an innate immune activating receptor expressed in humans mainly by natural killer (NK) cells but also by activated CD8⁺ T-cells, NK T-cells, and some CD4⁺ and $\gamma\delta^+$ T-cell subsets [10] and whose function is to recognize and kill infected or cancer cells. The NKG2D-based CAR format is reminiscent of a ‘first generation CAR’, however, the natural interaction between NKG2D and the endogenous co-stimulatory molecule DAP10 allows the CYAD-01 to function rather like a ‘second generation’ CAR (Fig. 1). In human, NKG2D targets 8 different stress-inducible ligands, expressed by a large variety of tumor cells, which provides CYAD-01 the potential to challenge a broad range of tumor indications [11–13]. In addition to the expected direct recognition of tumor cells, preclinical studies demonstrated that CYAD-01 also targets the non-tumor cells expressing NKG2D ligands within the stroma, tumor blood vessels and immunosuppressive TME, which allows for disruption of the essential support mechanisms required

for tumor cell survival and growth [14]. Furthermore, CYAD-01 targets several ligands from two main families potentially decreasing the likeliness of target loss and associated relapse after treatment which was often observed with single targeting CAR-T therapies. Finally, in the absence of patient preconditioning, CYAD-01 cells do not expand extensively *in vivo* and did not demonstrate long-term survival after infusion, whilst being able to induce tumor specific adaptive immunity. This is a particularly interesting safety advantage to prevent from strong side effects generally associated with rapid CAR-T expansion [15]. Collectively, this suggests that exploiting the targeting of NKG2D ligands by CYAD-01 would allow to treat diverse tumor indications with a single generic construct combining specificities of both innate and adaptive immunity, potentially providing a new paradigm for CAR T-cell therapy (Fig. 2).

Based on these unique properties of CYAD-01, Celyad initiated a trial with a tailored infusion schedule of CYAD-01 with reduced tendency of *in vivo* expansion and long-term persistence. The ongoing open-label THINK (THERapeutic Immunotherapy with NKR-2, NCT03018405) trial is a Phase I study which primarily aims to evaluate the safety and clinical activity of a multiple infusion schedule of the CYAD-01 treatment administered without prior preconditioning in refractory or relapsing patients with different cancer indications after failure of standard treatments. The study contains two segments: a dose-escalation segment which evaluates 3 different dose-levels of CYAD-01 (3×10^8 , 1×10^9 and 3×10^9 CYAD-01 per injection administered every two weeks) using a 3+3 design and an expansion segment which evaluates the recommended dose defined in the first segment, in each tumor type [14]. Dose-escalation is performed in two parallel segments evaluating separately hematological (acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), multiple myeloma (MM)) and solid tumor types (metastatic or locally advanced colorectal cancer (CRC), triple-negative breast cancer (TNBC), urothelial carcinoma, pancreatic cancer, recurrent ovarian carcinoma) indications. As of December 2017, two AML patients and 1 MM patient have received the CYAD-01 treatment at dose-level 1 (3×10^8 CYAD-01) and completed the safety period with no occurrence of dose-limiting toxicity (DLT). At the first dose-level (3×10^8 CYAD-01), a relapsed refractory AML patient reached a morphologic leukemia-free state (MLFS) and resolution of symptoms with the CYAD-01 treatment, with improved hemato-poiesis at 3 months follow up (Sallman et al., submitted for publication [16]). Within the solid tumor type cohort, 4 patients have received the CYAD-01 treatment at dose-level 1 (3×10^8 CYAD) and three patients have received CYAD-01 administrations at dose-level 2 (1×10^9 CYAD-01) and all completed the safety period with no DLT occurrence.

Moving forward the early clinical development of CYAD-01 in solid cancer indications by focusing on the challenges caused by the immunosuppressive TME, the SHRINK trial (Standard chemotherapy Regimen and Immunotherapy with NKR-2, NCT03310008) has been designed to evaluate the safety and clinical activity of a multiple infusion CYAD-01 treatment (3 infusions every 2 weeks) in patients receiving standard-of-care neoadjuvant FOLFOX (folinic acid, fluorouracil (5-FU) and oxaliplatin) chemotherapy regimen for the treatment of colorectal cancer with potentially resectable liver metastases. This administration of chemotherapy concurrently to the CYAD-01 treatment should not only favor modulation of and infiltration of the adoptively transferred CYAD-01 cells into the immunosuppressive TME but also allow better control of the disease due to the direct effect of chemotherapy on cancer cells, provide an opportunity for the CYAD-01 cells to better engraft due to the lymphodepletion induced by the standard chemotherapy administration, and likely increase the NKG2D ligand expression in tumor tissues targeted by CYAD-01, increasing the likeliness to

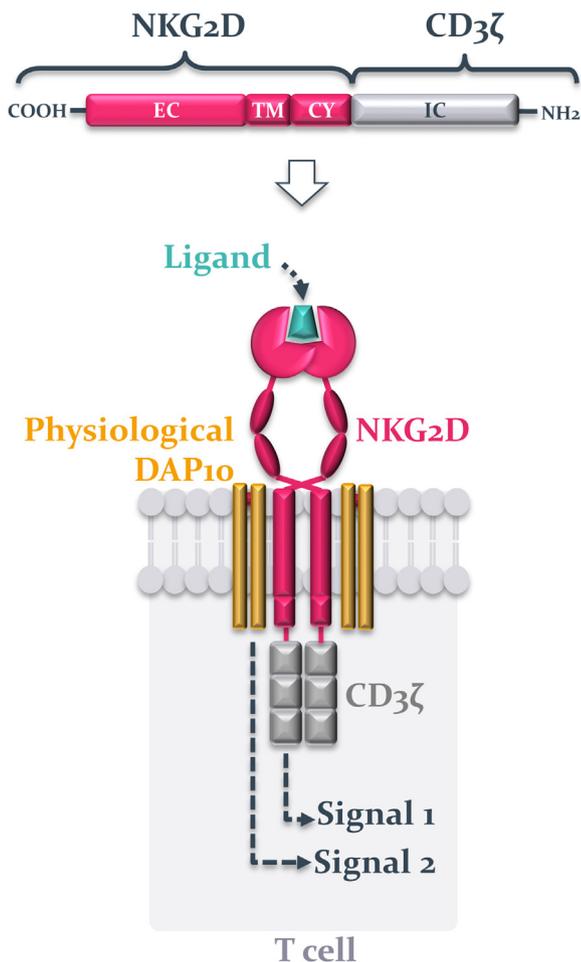


Fig. 1. The CYAD-01 construct. CYAD-01 construct contains the full-length human NKG2D receptor linked to the signaling domain of CD3 ζ that provides primary signaling (signal 1) which activates T-cells upon ligand binding. The adaptor molecule DAP10, naturally expressed in T-cells, provides for costimulatory signaling secondary signaling (signal 2) in conjunction with the primary signaling which allows CYAD-01 to actually work as a second-generation CAR-T. EC: extracellular domain, TM: transmembrane domain, CY: cytoplasmic domain, IC: intracellular domain.

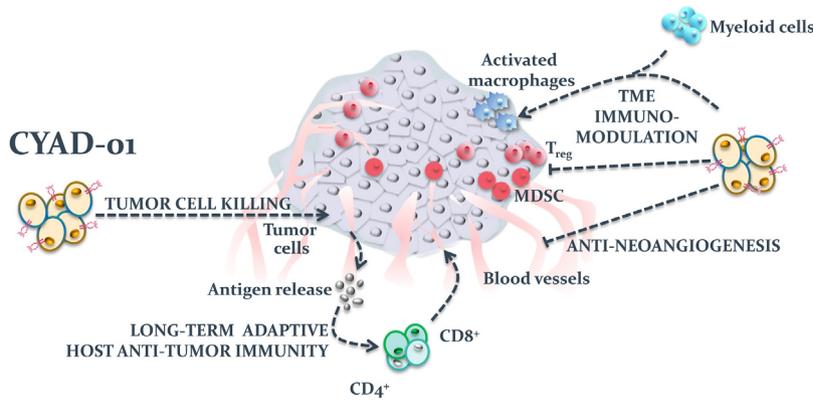


Fig. 2. CYAD-01 different mechanisms of action. Apart from direct cytotoxicity against cancer cells, CYAD-01 mode of action also involves modulation of the immunosuppressive tumor microenvironment, an anti-angiogenic activity and induction of a long-term anti-tumor specific memory immune response.

Table 1
Clinical development of CYAD-01 in solid tumors.

	THINK	SHRINK	LINK	DEPLETHINK
Assess safety and clinical activity of multiple doses of CYAD-01	+			
Classical CAR-T target product profile	+			+
Change CYAD-01 kinetics/dynamics				
• Increase CYAD-01 expansion and proliferation		+		+
• Favor loco-regional homing			+	
Increase CYAD-01 tumor infiltration		+		
Modulate the tumor immune microenvironment				
• Lift the immune-suppressive microenvironment		+		+
• Tumor inflammation		+	+	
Tumor targeting/antigenicity				
• Prime/favor adaptive immune response		+/-		+/-
• Increase NKG2D ligand expression		+		+
Tumor burden control and integration into standard treatment		+		
Safety profile (lower systemic toxicity)		↘	↗	↘

observe clinical benefits in patients presenting heterogeneity and/or low ligand expression.

To address the challenge related to the difficulty of CAR-T cells to access the site of metastasis, the LINK trial (Locoregional Immunotherapy with NKR-2, NCT03370198) has been developed to evaluate the safety and clinical activity of multiple hepatic transarterial administrations of the CYAD-01 treatment (3 infusions every 2 week) in colorectal cancer patients with unresectable liver metastases. The loco-regional hepatic transarterial administration (HTAA) cell therapy should lead to a persistent and increased concentration of the infused cells within the TME as well as a lower systemic toxicity as compared to systemic treatment. The difference in blood supply between uninvolved liver parenchyma and metastases may further favor CYAD-01 tumor homing. Moreover, based on the potential impact of the CYAD-01 treatment on the host immune system, combined with the tumor antigen spreading induced by its direct anti-cancer cells activity, the CYAD-01 HTAA might boost the adaptive immune response and therefore potentially control any distant lesion (abscopal effect).

Finally, to prolong durability of response, Celyad is considering evaluating the potential benefit of a preconditioning therapy prior to CYAD-01 T-cell injection in a future DEPLETHINK trial, to increase expansion and favor engraftment of CAR T-cells. One preconditioning therapy cycle (based on the classical CAR-T protocols *i.e.* a conditioning regimen consisting in 300 mg/m² cyclophosphamide and fludarabine 30 mg/m² daily for 3 days, followed 2 days later by injection of the CAR T-cells) will be therefore given prior to injection of CYAD-01. This non-myeloablative preconditioning chemotherapy regimen should allow a better proliferation and expansion of CAR T-cells due to the

lymphodepletion induced by chemotherapy (resulting in the eradication of some endogenous competing cells and the increased availability of homeostatic proliferative cytokines) and potentially improve the outcome of CYAD-01 therapy through modification of the immune microenvironment (usually responsible for the decreased efficiency of immunotherapies), but also, similar to standard chemotherapy, potentially increase the NKG2D ligand expression in tumor tissues targeted by CYAD-01.

Taken together, this breadth of clinical research activity investigating CYAD-01 CAR T-cell therapy (see Table 1) intends to test the concept of multiple stress ligand targeting by a single generic CAR construct and, hopefully, drive more intensive clinical examination of the NKG2D CAR T-cell approach.

Disclosure of interest

CL, NB, BV, DG and FL are employed by Celyad SA. PA declares no disclosures. JPM has advisory roles with Merck Sharp and Dohme (uncompensated), Innate Pharma, Debio, Boehringer-Ingelheim, Nanobiotix. The THINK, SHRINK and LINK clinical trials are sponsored by Celyad SA.

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