SPECIAL REPORT

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Exploiting natural killer group 2D receptors for CAR T-cell therapy

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Chimeric antigen receptors (CARs) are genetically engineered proteins that combine an extracellular antigen-specific recognition domain with one or several intracellular T-cell signaling domains. When expressed in T cells, these CARs specifically trigger T-cell activation upon antigen recognition. While the clinical proof of principle of CAR T-cell therapy has been established in hematological cancers, CAR T cells are only at the early stages of being explored to tackle solid cancers. This special report discusses the concept of exploiting natural killer cell receptors as an approach that could broaden the specificity of CAR T cells and potentially enhance the efficacy of this therapy against solid tumors. New data demonstrating feasibility of this approach in humans and supporting the ongoing clinical trial are also presented.

First draft submitted: 6 March 2017; Accepted for publication: 19 April 2017; Published online: 14 June 2017

Cancer immunotherapy

While surgery, chemotherapy and radiation remain the mainstays of current cancer therapy, exploiting the power of the immune system to target tumors (cancer immunotherapy) is emerging as a new paradigm to complement standard therapy. Engineering T cells by means of a chimeric antigen receptor (CAR) has recently provided highly encouraging results in patients with certain hematological cancers. CAR T cells are genetically engineered T cells displaying, at their cell surface, artificial receptors that recognize tumors expressing specific antigen(s) [1,2]. The targeting domain of this artificial receptor is generally coupled to an intracellular T-cell stimulatory domain (CD3 ζ) and depending on the CAR generation, one or two intracellular costimulatory domains (e.g., CD28 and CD137). Engagement of the extracellular domain of the CAR with its corresponding ligand triggers antigen-specific T-cell activation resulting in cytokine release and tumor cell killing. Most of the clinical data currently available describes CD19-specific CAR T-cell therapy for patients with B-cell malignancies, where many objective responses in early phase clinical trials have been observed. However, to date, CAR T-cell therapy in solid tumors has failed to achieve similar levels of clinical success [3–5]. There are many reasons that can explain poor efficacy in solid tumors, such as heterogeneous expression of the target, immunosuppressive microenvironment, lack or difficulty of access to the tumor, and poor trafficking of CAR T cells to the tumor site. In addition, the antibody-derived single chain antibody fragments (single chain fragment variable), typically used in classical CAR

KEYWORDS

- adoptive cell therapy
- CAR-T • chimeric antigen receptor • clinical trial
- CM-CS1 • immunotherapy
- NKG2D • NKR-2
- pancreatic cancer

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constructs only recognize one antigen. On one hand a single chain fragment variable provides a high specificity, but on the other hand, in a situation where the CAR T cell is not able to easily access its target, where the target is missing due to tumor heterogeneity or antigen escape mechanisms, effective therapeutic activity is challenging. As an alternative to these classical CAR limitations and to provide more options to target solid tumors, efforts are underway to exploit 'natural' receptors recognizing multiple antigens for CAR T-cell therapy.

• Natural killer group 2D protein to target T cells to solid & hematological tumors

Natural killer (NK) cells are part of the innate immune system and are naturally capable of killing tumor cells and virally infected cells. In addition, NK cells secrete cytokines that assist in

establishing an adaptive immune T-cell response and recruiting other immune cells, predominantly via interferon (IFN)- γ and tumor necrosis factor (TNF)- α secretion [6]. Their effector functions are tightly controlled by a balance between activating and inhibitory signals mediated by receptors at their surface [7].

To date, a well-characterized NK activating receptor is the NKG2D receptor, a C-type lectin-like, type II transmembrane glycoprotein. In humans, NKG2D is expressed on NK cells, NK T cells, CD8⁺ T cells and, under some conditions, in a subset of $\gamma\delta^+$ and CD4⁺ T cells [8,9]. To achieve expression at the cell surface, NKG2D associates with an adaptor molecule called DNAX-activating protein of 10 kDa (DAP10). The cytoplasmic domain of DAP10 actively participates in cell activation since it is phosphorylated upon interaction of NKG2D with its

Figure 1. Comparison between classical chimeric antigen receptor and NKR-2. Classical CAR T cells are composed by an antigen-specific antibody-like ectodomain (single chain fragment variable) fused to a hinge, a transmembrane domain, an intracellular activating domain (CD3 ζ) and, depending on the CAR generation, to one or two costimulatory domains. NKR-2 uses the NKG2D complex to provide primary and costimulation signals: the full length NKG2D protein (ectodomain, transmembrane and intracellular domains) is fused with an inverted intracellular domain of CD3 ζ . Moreover, DAP10, that stabilizes the complex, also triggers a costimulatory signal that makes NKR-2 a second-generation CAR. By contrast to a classical CAR specific for one target, the NKG2D ectodomain recognizes multiple ligands and allows the potential recognition of many tumor types. CAR: Chimeric antigen receptor.

Figure 2. NKR-2 T cells secrete IFN- γ when cultured in presence of NKG2D ligand-expressing cancer cell lines. Human T cells retrovirally transduced with the truncated form of CD19 (tCD19) or with the NKR-2 construct containing the tCD19 tag were incubated for 24 h, 1:1 E:T ratio, with cancer cell lines expressing NKG2DL: HCT-116 (human colon tumor cell line); K562 (human chronic myelogenous leukemia); PANC-1 (human pancreatic tumor cell line) and RPMI8866 (human leukemic cell line). A NKG2DL-negative cancer cell line, P815 (a murine mastocytoma cell line) was also included. After 24 h of incubation, cell-free medium was harvested and tested for IFN- γ by ELISA. Mock tCD19 T cells were not activated while incubated with cancer cell lines. On the contrary, NKR-2 transduced T cells secreted large amounts of IFN- γ when incubated with NKG2DL-positive cell lines but not with P815 cells indicating that NKR-2 T cells require NKG2DL expression on tumor cell lines. Results are the mean $\pm$ SD of triplicates, and data are representative of four to six experiments.

ligand. Then DAP10 recruits and activates the p85 subunit of phosphoinositide-3-kinase and the growth factor receptor-bound protein 2/vav guanine nucleotide exchange factor 1 complex, which triggers downstream signaling cascades leading to effector activities [10,11].

In humans, two families of NKG2D ligands (NKG2DL) have been identified, the MHC class I chain related proteins A (MICA) and B (MICB) and the structurally diverse unique long 16 binding proteins, in other words, UL16-binding proteins 1 to 6 (ULBP1–6). NKG2DL expression is tightly regulated by transcriptional, translational and post-translational mechanisms in healthy adult tissues to prevent self-recognition and autoimmune reactivity [12]. However, cell surface protein expression may be induced by various stress situations, such as viral infection, oxidative or thermal stress, genotoxic drugs, tissue damage, heat shock, inflammation (e.g., autoimmune diseases) or malignant transformation [12,13]. Accordingly, many tumor cell lines and primary tumors from diverse tissue origins express NKG2DL (see Table 1) implying NKG2DL to be putative targets on both solid and hematological tumors [14,15].

NKG2D CAR T cells

In 2005, Professor Charles Sentman's team tested the fusion of the full length NKG2D protein with CD3 ζ as a CAR expressed in primary T cells [16]. At first sight, this NKG2D-CD3 ζ fusion construction is built as a first-generation CAR (Figure 1). However, as mentioned earlier, NKG2D's natural binding partner DAP10 also triggers a costimulatory signal while NKG2D recognizes its ligands [17–19]. Thus, the NKG2D-CD3 ζ CAR initiates both activation signal 1 and signal 2 upon ligand binding in a manner akin to a second-generation CAR. With this design, the NKG2D-CD3 ζ CAR takes advantage of the configuration of the NKG2D receptor complex with two NKG2D molecules and four DAP10 molecules.

• Preclinical development

Over the last decade, the Sentman laboratory has carried out an extensive analysis of the *in vitro* functional activity of T cells expressing the NKG2D-CD3 ζ CAR (hereafter termed chimeric NKG2D [chNKG2D] when it is referring to the murine NKG2D CAR, and NKR-2 when it is the human NKG2D CAR) on cancer cell lines as

well as *in vivo* experiments in mouse tumor models. Preclinical data generated with chNKG2D T cells derived from murine cells demonstrate the huge potential of this technology to target solid and hematologic tumor types in immunocompetent syngeneic murine models of ovarian cancer [20], melanoma [21], lymphoma [22] and multiple myeloma (MM) [23]. In addition to this previous work, we report here new data demonstrating, for the first time, that human NKR-2 T cells are effective *in vitro* and *in vivo* in a mouse xenograft model of human pancreatic cancer.

As shown in Figure 2, *in vitro* coculture of human NKR-2 T cells with human cancer cell lines from multiple origins provided valuable information. First, NKR-2 T cells released IFN- γ upon coculture with a colorectal cancer cell line (HCT-116), a pancreatic cancer cell line (PANC-1) and leukemia cell lines (K562 and RPMI8866) all expressing NKG2DL, therefore confirming the work previously done [24,25] and extending the range of cancers potentially treatable with NKR-2. Second, NKR-2 T cells were not activated by cells not expressing human

Figure 3. NKR-2 T cells display cytotoxic activity against NKG2D ligand-positive cancer cell lines. PANC-1, HCT-116 and K562 stably expressing luciferase were cocultured at various E:T ratios with human T cells retrovirally transduced with tCD19 or with NKR-2. After 24 h, the bioluminescent signal corresponding to remaining viable tumor cells was quantified. (A) Co-incubation of luciferase expressing PANC-1 cells with NKR-2 T cells led to a loss of tumor cells as the E:T ratio increased. At 10:1 ratio, only 23% of PANC-1 survived to NKR-2 cytolytic activity while 96.1% of cells were still alive in the mock condition. (B) Co-incubation of luciferase expressing HCT-116 cells with NKR-2 T cells led to a decrease in luminescence signal even at the lowest ratio (0.5:1) where 68% of HCT-116 cells were already eliminated. (C) Co-incubation of luciferase expressing K562 cells with NKR-2 T cells led to a similar decrease in luminescence signal even at the lowest E:T ratio (-35%). Tumor cells alone = 100% luminescence. Results represent mean $\pm$ SD of triplicates, and they are representative of two or three experiments.

Figure 4. NKR-2 T cells inhibit PANC-1 progression in NOD-scid IL2Rgamma^{null} mice. NSG mice were injected IP with 0.5×10^6 luciferase-expressing PANC-1 tumor cells and tumor engraftment was followed by bioluminescent imaging ($n = 1$). Mice in all treatment groups, except for mouse number 4 from mock tCD19 group (for which tumor injection failed), showed strong luminescence signal from the PANC-1 cells in the abdominal cavity at the first imaging prior to T cell injection (day 7), showing significant tumor burden. Twelve and nineteen days after PANC-1 injection, control mice received either HBSS (HBSS group), 5×10^6 of T cells (IP) retrovirally transduced with the tCD19 mock construct (mock-tCD19 group) or with the NKR-2 construct (NKR-2 group). Imaging was performed every week starting one week after tumor inoculation. The tumor cell luminescence was noticeably decreased and more localized in mice that received the NKR-2 T cells. Thirty-five days post tumor injection, the final imaging showed that some mice that received the NKR-2 treatment had little tumor remaining compared with HBSS and mock T cell groups where several mice had to be euthanized and the others had a large tumor burden. The *in vivo* efficacy of NKR-2 cells against PANC-1 cells was further confirmed in NSG mice IP injected with luminescent PANC-1 and IV treated with NKR-2 ($n = 2$, data not shown).

IP: Intraperitoneally; IV: Intravenously; NSG: NOD-scid IL2Rgamma^{null}.

NKG2DL (the murine mastocytoma cell line P815), consistent with NKR-2 T cells secreting IFN- γ when the target cells express NKG2DL hence decreasing the risk of potential 'off-target, off-tumor' toxicity issues.

The potential of the NKR-2 technology was further confirmed by demonstrating the *in vitro*

cytolytic activity of NKR-2 T cells towards human NKG2DL-expressing cancer cell lines (Figure 3). Several previous studies showed that NKR-2 T cell lysis of tumor cells is dependent of NKG2D and NKG2DL [24,26]. Luciferase-expressing tumor cell lines (PANC-1, HCT-116 and K562 cells) were co-incubated 24 h with

NKR-2 T cells at different Effector:Target (E:T) ratios. A decrease of the bioluminescence signal was indicative of fewer tumor cells thus indicating specific killing of these tumor cells by NKR-2 T cells. As shown in [Figure 3](#), the coculture of luciferase-expressing tumor cell lines with NKR-2 T cells led to a decrease in bioluminescence signal in a manner dependent of the E:T ratio compared with mock T cells (transduced with a retroviral vector only coding for the truncated form of human CD19). At the highest E:T ratio, the mock T cell cocultures showed some decreased luciferase signal, however NKR-2 T cell cocultures displayed a much lower level. Collectively, these results confirm that human NKR-2 has the potential to target multiple cancer indications.

The *in vivo* antitumor efficacy of human NKR-2 T cells in a xenograft model of human pancreatic cancer in NOD-scid IL2Rgamma^{null}

(NSG) immunodeficient mice was then performed. As shown in [Figure 4](#), intraperitoneal (IP) treatment of established PANC-1 tumors with human NKR-2 T cells led to a strong reduction of PANC-1 tumor growth. This diminution of the tumor mass was not observable in mice treated with mock T cells or HBSS (vehicle control). Moreover, additional experiments where NKR-2 T cells were intravenously (IV) injected showed decreased tumor growth compared with mock T-cell controls (data not shown). This suggests an efficient trafficking of NKR-2 T cells to the tumor site in this model. These results, in addition to those already published [20–25], provide a strong rationale that NKR-2 T cells can effectively target a range of established tumors, and can even lead to complete tumor eradication in many situations. This particularly supports the development of a clinical trial in pancreatic cancer. Moreover,

Table 1. NKG2D ligands expression on various cancer.		
Tumor type	Expressed NKG2D ligand	Ref.
Acute lymphoblastic leukemia	28–67% MICA/B 9–20% ULBP1–3	[45–51]
Acute myeloid leukemia	0–75% MICA/B 16–50% ULBP1 4–64% ULBP2 16–100% ULBP3	[45,46,49,50,52–62]
Bladder carcinoma	70% MICA	[63,64]
Brain cancer	90% MICA, MICB and ULBP1–3	[65–67]
Breast cancer	35–100% MICA/B, ULBP 1–5	[68–72]
Cervical cancer	20% MICA, ULBP2	[73,74]
Chronic lymphocytic leukemia	0–85% MICA/B 10–20% ULBP1–3	[46,49,50,75,76]
Chronic myeloid leukemia	28–100% MICA/B 12–20% ULBP1–3	[46,50,77,78]
Colorectal cancer	80–100% MICA/B ULBP 1–5	[68,79,80]
Gastric carcinoma	40–100% MICA/B, ULBP2	[81–83]
Hepatocellular carcinoma	60–100% MICA	[84–87]
Lymphoma	28–44% MICA/B 12–20% ULBP1–3	[48,50,51,88–95]
Melanoma	50% MICA/B	[96–99]
Multiple myeloma	10–60% MICA 0–34% ULBP1–3	[36,100–105]
Neuroblastoma	86% MICA/B, ULBP1–3	[106]
Non-small-cell lung carcinoma	20–30% MICA/B, ULBP1–3	[68,107–109]
Ovarian carcinoma	50–97% MICA/B, ULBP1–5	[68,110–113]
Pancreatic cancer	68–89.3% MICA/B	[114–116]
Prostate cancer	75–95% MICA/B, sMICA/B	[68,117]
Renal cell carcinoma	> 95% MICA/B	[68,78,118,119]
Sarcoma	100% MICA/B, ULBP 1–3	[120,121]
MICA/B: MHC class I chain related proteins A and B; ULBP: UL16-binding proteins.		

as shown in syngeneic tumor models, animals that have eliminated tumors can resist to rechallenge with the same tumor cells without additional therapy [20]. Since no murine chNKG2D T cells were detected prior to rechallenge and host lymphocytes were able to now recognize the tumor, these findings indicate that the initial chNKG2D therapy induced a long-term memory immune response against unknown tumor antigen(s) by activating the immune system of the host.

In addition, and by contrast to a classical CAR, chNKG2D efficacy is independent of lymphodepleting preconditioning [23]. Moreover, chNKG2D was shown to be the most effective when cells were administered multiple times. Indeed, a single chNKG2D dose was shown to double the overall survival of treated mice compared with control; nevertheless, three doses were necessary to reach complete survival in a lymphoma model [22]. Interestingly, chNKG2D murine T cells also demonstrated antitumor efficacy on ovarian tumors composed of a mix of NKG2DL-negative and -positive tumor cells. chNKG2D T cells were still able to reduce ovarian tumor progression in tumors having 93% of NKG2DL-negative cells [27]. This strongly suggests that NKR-2 cells may be effective even in highly heterogeneous tumors. Together, this makes the NKR-2 approach very different than anti-CD19 CAR T cell therapy where a combination of a preconditioning chemotherapy with single dose of CAR T cells given shortly afterward remains essential for the success of the therapy [28].

Beyond this direct antitumor activity, murine models showed a change in the tumor microenvironment of animals receiving chNKG2D T cells. Indeed, while the immunosuppressive immunosuppressive T regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSC) usually impair the cytotoxic ability of NK and T cells, it was shown that tumor-derived MDSCs [29] or Tregs [30,31] express NKG2DL and may be targeted by NKG2D-specific effector cells. Thus, NKR-2 putatively triggers a shift towards a pro-inflammatory environment and amplifies the natural immune system response to eradicate tumors.

Interestingly, there was also evidence of expression of NKG2DL on endothelial cells found in tumor neovasculature that supported observations of antitumor response in tumors

that lack the expression of NKG2DL on their tumor cells [21].

Finally, as a potential mechanism of tumor immune escape, NKG2DL can be shed from the membrane of tumors cells and can even be measured in sera of cancer patients (0–15 ng/ml) [32]. The impact of these soluble NKG2DL (sNKG2DL) in the context of NKR-2 is still unknown. sNKG2DL have been shown to downmodulate NKG2D expression on the surface of NK cells and inhibit NK cell and T-cell functions *in vitro* [33–35]. Therefore, these data suggest that sNKG2DL may locally reduce recognition of tumor cells by diminishing NKG2DL densities at the cancer cell surface, but may also systematically impair NKG2D receptor function in cytotoxic immune effector cells. However, Zhang *et al.* showed that recombinant soluble MICA (at a concentration of as high as 1.5 µg/ml) did not interfere with *in vitro* killing activity of NKR-2 T cells toward different tumor cell lines (K562 and RPMI8866). This suggests that sMICA in cancer patients may not inhibit the function of NKR-2 T cells *in vivo* [24]. Moreover, von Lilienfeld-Toal *et al.* showed that clinically relevant concentrations of sNKG2DL did not downregulate NKG2D expression *in vitro* [36]. Although we cannot exclude that levels of sNKG2DL at the tumor site could be different from the one measured in the serum, the fact that neither NKG2D expression nor NKG2D activity is affected by very high concentrations of sNKG2DL suggests that sNKG2DL should not interfere with NKR-2 T cell activity *in vivo*.

Taken together, direct antitumor activity, the potential effect on tumor neovasculature, the tumor microenvironment modulation, the putative insensitivity to sNKG2DL and the potential ability to target eight different ligands makes NKR-2 T cell a candidate that has the capacity to go beyond what is currently available for the ‘standard’ CAR T-cell approach.

• Clinical development

Since preclinical studies have shown the therapeutic potential of the murine chNKG2D T cells in both hematologic and solid tumor types and since the expression of NKG2DL was demonstrated on various solid cancer types, early clinical development programs started with CM-CS1 and THINK clinical trials.

CM-CS1: initial clinical testing of NKR-2

A Phase I clinical study (CM-CS1) to establish the safety and feasibility of a single infusion of NKR-2 T cells without lymphodepletion started in April 2015 and was completed for enrollment in August 2016. Twelve patients with acute myeloid leukemia (AML)/myelodysplastic syndrome refractory anemia with excess blasts (MDS-RAEB), or relapsed/refractory progressive MM without standard therapy options were infused with increasing doses of NKR-2 T cells (NCT02203825). The dose escalation involved four cohorts of patients starting at a low initial single dose of 10^6 cells up to 3×10^7 NKR-2 cells per patient. The low doses reflect a prudent clinical development plan due to the novelty of the target. The initial clinical data presented a good safety profile since neither sign of cytokine release syndrome, nor tumor-lysis syndrome or off-tumor on-target toxicity was observed. In addition, pre-clinical observations were corroborated since a short-term persistence of the NKR-2 cells in the peripheral circulation has also been observed. There were no objective responses by the day 28 evaluation. However, preliminary observations of unexpected survival and/or improvement in hematologic parameters were noted in both AML and MM patients with or without subsequent therapy as reported at the American Society of Hematology Annual meeting [37].

THINK: expanding clinical investigation of NKR-2 in solid & hematological tumors

The CM-CS1 clinical trial confirmed the initial safety and tolerability of a single dose of NKR-2 T cell. The target product profile of NKR-2 is derived from preclinical models involving three administrations of the NKR-2 product, 2 weeks apart [20,22]. Therefore, the THINK trial (EudraCT number 2016-003312-12, NCT03018405) has been devised to test NKR-2 cell therapy further in the two hematological indications examined in CM-CS1 (MM, AML) and also in five solid tumor types (ovarian, colorectal, pancreas, bladder and triple-negative breast cancers). The THINK trial involves a dose escalation starting with three doses of 3×10^8 NKR-2 cells up to three doses of 3×10^9 cells, each dose being administered every 2 weeks. The primary objective of the THINK trial is safety and early signs of clinical efficacy. Data presented in this special report (Table 1 & Figures 2–4) confirm that pancreatic tumor cells

are targeted by human NKR-2 T cells *in vitro* and *in vivo* and support the ongoing solid tumor trial that opened to recruitment in January 2017 and is expected to be completed in mid-2018.

NKR-2 T cells display many potential advantages as compared with the ‘standard’ CAR T cell therapy. Multiple ligand targeting, lack of patient’s preconditioning, induction of tumor specific adaptive immunity, anti-neoangiogenesis potential, activity even in NKG2DL heterogeneous tumors and modulation of the tumor microenvironment are combined to provide what could be an ideal therapeutic approach. However, preclinical mouse models rarely accurately predict responses in patients with advanced cancers. A specific concern is on-target/off-tumor activity and the multiple targeting capacity of stress-induced ligands by NKR-2. Pre-clinical models indeed suggest that very high doses of chNKG2D murine T cells infused as a bolus can induce toxicity in the mouse [38]. Similarly, preconditioning chemotherapies, combined with high doses of chNKG2D murine T cells, were shown to induce lethal toxicity in mice [39]. All these elements underscore the approach being taken in the Therapeutic Immunotherapy with NKR-2 (THINK) trial. The multiple dosing approach avoids reaching high doses where such toxicity is observed and allows a higher number of total cells to be given over a prolonged period.

Future perspective

In theory, the efficacy of a variety of different effector cells could be enhanced using the NKR-2 technology (CIK, NKT and NK cells) [40–42]. A group has published that NK cells transduced to express a chimeric NKG2D have a greater cytotoxicity [26]. Therefore, NKR-2 may also allow other effector cell types to recognize NKG2D ligands and makes them more effective against tumors. Whether NKR-2 T cells alone will be able to deliver a sufficient ‘hit’ against an established tumor in humans remains to be determined. However, the absence of preconditioning and the multiple dosing regimen currently used in the THINK trial provides an entirely suitable approach that allows combination with other therapies. One of the potential combinations would be with checkpoint inhibitors that have already shown some clinical benefits in solid tumors as a monotherapy [43]. The leverage of the immune brake by checkpoint inhibitors combined with

NKR-2 could be a powerful solution to reach clinical efficacy. Alternatively, ionizing radiations are known to trigger NKG2DL expression on various cancer cell lines [14,44], making the combination of this standard therapy with NK-2 a potential association that might increase NK-2 potency. Similarly, technologies enhancing NK-2 T cells trafficking to tumors or modulating the tumor micro-environment (metabolically or immunologically) are all potential combinations for NK-2. How NK-2 may be optimally combined with such therapies remains to be established. However, the multiple modes of action of NK-2 provides a potential paradigm shift where CAR T cells and the induction of adaptive immunity together may become a game changer for the future of immuno-oncology.

Financial & competing interests disclosure

B Demoulin is an employee of Celyad SA. J Muradis is an employee of Celdara Medical. ML Sentman is related to a patent owner of this technology and is a consultant for Celdara Medical and Celyad. C Loney is an employee of

Celyad, SA. D Gilhamis an employee of Celyad. CL Sentman is a Scientific Founder and consultant of Celdara Medical, LLC, holds patents on this technology and is a consultant for and has a sponsored research agreement with Celyad, SA. S Agaugue is an employee of Celyad SA. All this work was done under a Sponsored Research Agreement funded by Celyad SA. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct

All animal work was performed at the Geisel School of Medicine Animal Facility in accordance with institutional guidelines.

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EXECUTIVE SUMMARY

NKR-2 chimeric antigen receptor T cells

- NK-2 chimeric antigen receptor (CAR) T cells express a chimeric construct created by fusing the full length NKG2D protein with the CD3ζ intracellular domain. This construct is built as a first-generation CAR but act as a second-generation CAR due to DAP10 costimulatory signaling.

Preclinical development

- Murine chNKG2D T cells are efficient *in vitro* and *in vivo* against several solid and liquid tumors such as ovarian cancer, melanoma, lymphoma and multiple myeloma.
- New data presented in this special report demonstrate the *in vitro* antitumor efficacy of NK-2 on colorectal and pancreatic cancer cell lines.
- In a model of xenograft of human pancreatic cancer, we demonstrate, for the first time, the *in vivo* antitumor efficacy of NK-2 against an established pancreatic cancer.
- In addition to direct efficacy against a wide range of tumor cell lines or established tumors, preclinical data show that murine chNKG2D T cells:
 - Induce a long-term memory immune response against tumor antigen(s);
 - Are effective without lymphodepletive preconditioning;
 - Are effective against heterogeneous tumors;
 - Impact tumor microenvironment by targeting immune-suppressive cells and neovasculature.

Clinical development

- The CM-CS1 clinical trial demonstrates the safety and feasibility of a low single-dose infusion of NK-2 cells in hematological cancers.
- THINK trial expands clinical investigation made in CM-CS1 by testing multiple and higher doses. This trial is design to test NK-2 in two hematological indications (acute myeloid leukemia and multiple myeloma) and in five solid cancers (ovarian, colorectal, pancreas, bladder and triple-negative breast cancers).

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