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Expression of a TIM peptide reduces alloreactivity of T cells facilitating an allogeneic NKG2D Chimeric Antigen Receptor T cell therapy approach

Alexandre Michaux[1], Eytan Breman[1], Sébastien Mauen[1], Jennifer Bolsée[1], Laura Saerens[1], Benjamin Violle[1], Fanny Huberty[1], Jérôme Marijsse[1], Céline Jacques-Hespel[1], Céline Marchand[1], Nancy Ramelot[1], Thuy Nguyen[1], Julien Houssa[1], Charles L. Sentman[2], David Gilham[1], Sophie Agaugué[1]

[1]*Celyad, Mont-Saint-Guibert, Belgium*
[2]*Geisel School of Medicine at Dartmouth, Lebanon, NH, USA*

BACKGROUND

Chimeric antigen receptor (CAR) T cells have shown impressive clinical results especially in B cell malignancies. Most CAR T cell therapy relies on autologous patients' cells which may have limitations in terms of patient T cells manufacturing. T cells derived from an allogeneic healthy donor may circumvent some of these issues. However, allogeneic T cells can induce graft versus host disease (GvHD), a response triggered by the recognition of non-self Human Leukocyte Antigen molecules expressed on recipient cells by the T Cell Receptor (TCR) of donor cells.

To interfere with TCR signaling and avoid GvHD, a TIM (TCR inhibitory molecule) peptide consisting of a truncated form of CD3ζ was generated. To assess TIM in the context of a CAR therapy, an allogeneic CAR T cell (referred as CYAD-101) was developed by co-expressing TIM and a NKG2D-based CAR (NK2)

RESULTS

TIM and allogeneic T cells process

The octameric TCR/CD3 complex is formed by the association of the variable antigen-binding TCRα/β heterodimer with the CD3 signaling machinery (CD3γ/ε, CD3δ/ε and CD3ζ/ζ dimers). TIM (TCR inhibitory molecule) is a shortened form of human CD3ζ truncated before the first ITAM (Immunoreceptor Tyrosine-based Activation Motif) sequence, depriving this peptide of any signaling capability when incorporated into the TCR/CD3 complex (**Figure 1**). Celyad developed an allogeneic approach based on this TIM technology. Briefly, PBMCs from an healthy donor are activated with OKT3 before transduction with retroviral vector and purification using a transduction marker (i.e. a truncated form of CD19, tCD19). tCD19 alone refers as our mock construct on this presentation. Purified cells are then seeded for expansion (**Figure 2**).

TIM expression in T cells impairs allogenicity *in vitro* and *in vivo* by altering TCR activation pathways

Impact of TIM on TCR activation was assessed *in vitro* on both tCD19 mock and hT8.1 (TIM vector) transduced T cells (**Figure 3**). Those T cells were incubated in the presence of increasing concentrations of the αCD3ε antibody OKT3 (0-200 ng/ml) for 24h hours. OKT3 induces a non-HLA-restricted antigen-independent T cell activation. Interestingly, we observed an inhibition of the TCR-mediated activation of around 50% as reflected by reduced IFN-γ production. Preliminary results showed that this impairment was due to a decrease of TCR activation pathways in TIM transduced cells. Phosphoproteins quantification presented on **Figure 4** shows an overall decrease in the abundance of 4 major actors of the TCR activation pathway (*i.e.* ZAP-70, ERK, JNK and NF-κB) in TIM transduced cells upon TCR activation. Interestingly, the impairment of allogenicity in TIM transduced T cells observed *in vitro* was confirmed *in vivo* since TIM expression suppressed xenoGvHD in NSG mice while mock tCD19 T cells were not (**Figure 5**) .

FIGURES & TABLES

FIGURE 1: TCR inhibitory molecule (TIM), TCR/CD3 complex and NKR2

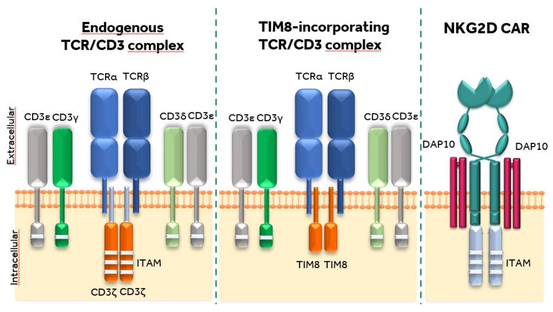


FIGURE 2: production process of allogeneic TIM8-NKR2

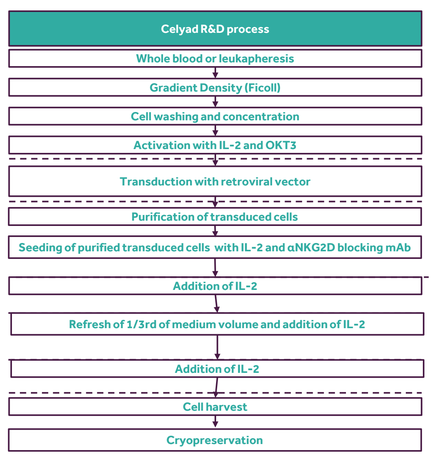


FIGURE 3: TIM expression in T cell decreases cytokine production upon OKT3-mediated TCR activation

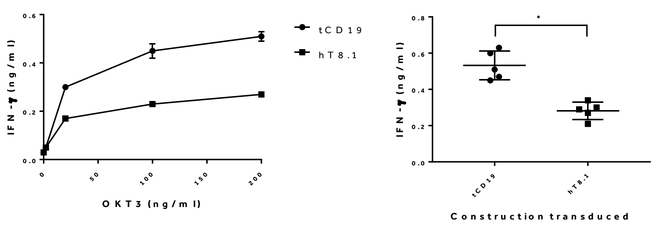


FIGURE 4: Preliminary data suggest that TIM expression impacts TCR activation pathway

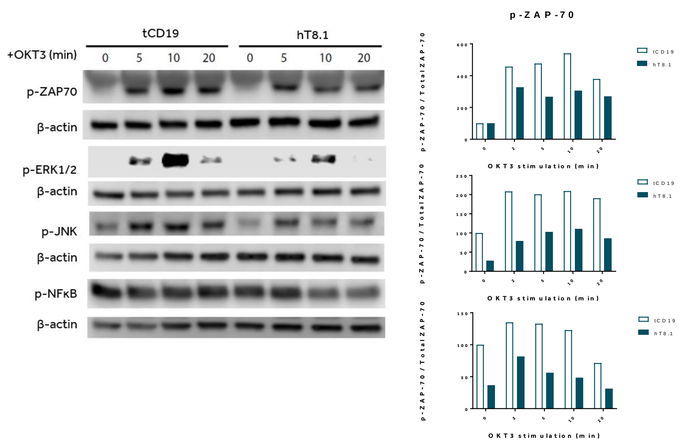


FIGURE 5: Inhibition of GvHD onset by TIM8-expressing T cells

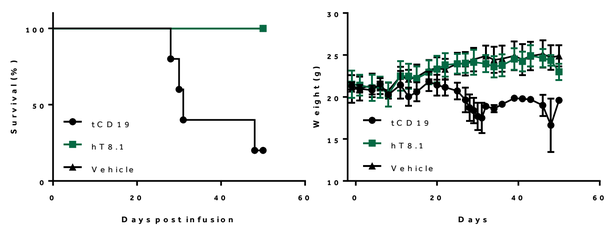


FIGURE 6: CYAD-101: an allogeneic CAR T cell co-expressing TIM and a NKG2D-based CAR.

FIGURE 7: CYAD-101 cells show decreased TCR surface expression and TCR activation compared to control alloreactive T cells

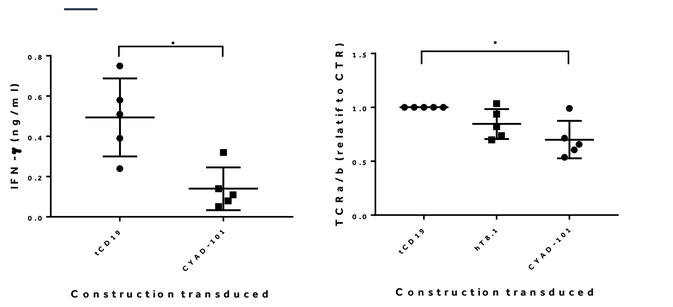


FIGURE 8: CYAD-101 T cells do not induce any GvHD in NSG mice by contrast to control alloreactive T cells

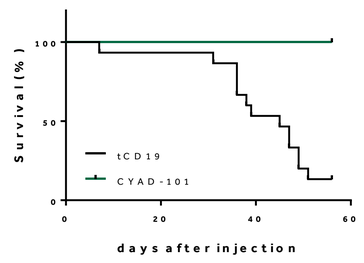


FIGURE 9: CYAD-101 is potent against tumour cell lines *in vitro*

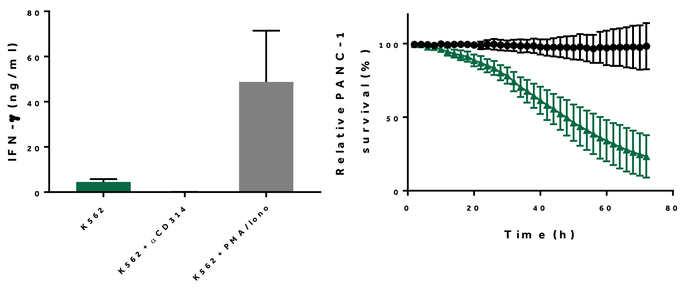


FIGURE 10: 3 IV injections of CYAD-101 cells improve significantly survival of NSG mice implanted orthotopically with a colorectal HCT-116 tumor

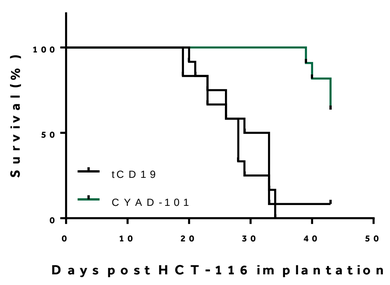
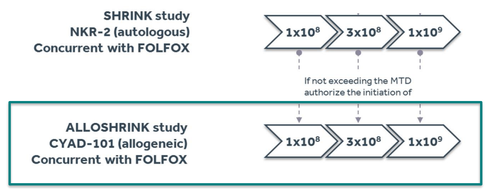


FIGURE 11: Clinical development of CYAD-101



RESULTS

CYAD-101 is a good candidate for allogeneic CAR T cell therapies

CYAD-101 was developed by co-expressing TIM and a NKG2D-based CAR (illustrated on **Figure 1 and 6**). The NKG2D CAR is composed of the full-length human NKG2D fused to the ITAM-bearing signaling cytoplasmic domain of human CD3ζ to allow NKG2D to function as a primary receptor in T cells. Together NKG2D-CAR and its endogenous partner DAP10 create a new receptor complex which enables T cells to recognize and kill tumor cells expressing NKG2D ligands. The allogenicity of CYAD-101 was confirmed by no or low IFN-γ production after TCR-mediated activation with increasing doses of OKT3 (**Figure 7**). Interestingly, CYAD-101 cells showed a significative decrease in TCRα/β surface expression compared to mock cells when stained by flow cytometry. Inhibition of GVHD induction by CYAD-101 was confirmed *in vivo* (**Figure 8**). NSG mice receiving mock T cells succumbed to GvHD with a median survival of 47 days (range 7 – 51 days) . By comparison, none of the mice injected with CYAD-101 cells succumbed before the end of the experiment (day 56).

To assess the *in vitro* functionality of NKG2D CAR in a TIM context, CYAD-101 was challenged with the NKG2D-ligand positive human cancer cell line K562 (chronic myeloid leukemia) in the presence or absence of αNKG2D Ab (5µg/mL). After 24 hours of coculture, cytokine release was analyzed by ELISA (IFN-γ). As shown in **Figure 9**, CYAD-101 cells produced high amounts of IFNγ in the presence of K562 cells while this production was completely inhibited in the presence of αNKG2D blocking Ab, demonstrating the specificity of the recognition of NKG2D ligands by CYAD-101 cells.

The kinetics of CYAD-101 cytolytic activity against PANC-1 cells (pancreatic cancer) was further assessed using the IncuCyteTM System. Briefly, cryopreserved CAR T cells were cocultured with PANC-1 cells stably expressing a nuclear restricted red fluorescent label. As negative control, PANC-1 cells were cultured with mock tCD19 T cells. Red fluorescence and as such, living PANC-1 cells were monitored every 2 hours for 72 hours and showed a reduction of 80 % the living PANC-1 cells after 72h when cultured with CYAD-101. This potency of CYAD-101 against tumors was confirmed *in vivo* since 3 injections of CYAD-101 cells delayed the growth of an orthotopic HCT116 (colorectal cancer (CRC)) tumor in the NSG mouse model (**Figure 10**).

The further development of CYAD-101 will consist in the AlloSHRINK trial, an open-label. This protocol consist in a phase I study to assess the safety of multiple doses of CYAD-101sts in 3 injections with a 2-week interval between each administration in metastatic CRC, concurrently with a standard chemotherapy treatment.

RELEVANT LITERATURE:

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CONCLUSIONS & PERSPECTIVES

T cells modified with the TIM showed a significant reduction in IFN-γ secretion after mitogenic stimulation when compared to control-alloreactive T cells. Moreover, no xeno-GvHD was observed in NSG mice treated with human TIM T cells. Preliminary results indicate that TIM acts as a dominant negative form of CD3z inhibiting several actors of the TCR signaling pathway.

When TIM was co-expressed with an NKG2D specific CAR (CYAD-101), CYAD-101 cells displayed reduced allo potency whilst maintaining potentNKG2D- specific invitro and in vivo anti-tumor activity against established orthotopic colorectal tumors confirming CYAD-101 as an attractive allogenic CART cell therapy.

The clinical development of CYAD-101 will be further pursued in a phase I trial to assess the safety and the clinical activity of multiple doses administered with standard chemotherapy in patients with unresectable metastatic CRC.