Chimeric Antigen Receptor Therapy in Haematology and Oncology: Current Successes and Challenges

Treatment of metastatic renal cell carcinoma (mRCC) with CAIX CAR-engineered T-cells – a completed study overview

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Abstract

We studied safety and proof of concept of a phase I/II trial with chimeric antigen receptor (CAR) T-cells in patients with metastatic renal cell carcinoma (mRCC). The CAR was based on the G250 mAb that recognized an epitope of carboxy-anhydrase-IX (CAIX). Twelve patients with CAIX + mRCC were treated in three cohorts with a maximum of 10 daily infusions of 2×10^7 to 2×10^9 CAR T-cells. Circulating CAR T-cells were transiently detectable in all patients and maintained antigen-specific immune functions following their isolation post-treatment. Blood cytokine profiles mirrored CAR T-cell presence and *in vivo* activity. Unfortunately, patients developed anti-CAR T-cell antibodies and cellular immune responses. Moreover, CAR T-cell infusions induced liver enzyme disturbances reaching CTC grades 2–4, which necessitated cessation of treatment in four out of eight patients (cohort 1 + 2). Examination of liver biopsies revealed T-cell infiltration around bile ducts and CAIX expression on bile duct epithelium, adding to the notion of on-target toxicity. No such toxicities were observed in four patients that were pretreated with G250 mAb (cohort 3). The study was stopped due to the advent of competing treatments before reaching therapeutic or maximum tolerated dose in cohort 3. No clinical responses have been recorded. Despite that, from this trial numerous recommendations for future trials and their immune monitoring could be formulated, such as choice of the target antigen, format and immunogenicity of receptor and how the latter relates to peripheral T-cell persistence.

Introduction

Adoptive transfer of gene-modified T-cells equipped with either chimeric antigen receptors (CARs) or T-cell receptors (TCRs) provides an attractive strategy to provide therapeutic immunity against malignancies. In recent years, CAR T-cell therapy has shown impressive clinical responses in haematological B-cell malignancies [1,2], whereas gene-modified T-cells so far failed to yield anti-tumour responses in a substantial number of patients in solid tumours [3–7].

Improved insight into several aspects is urgently needed in order to fully explore the potential of gene-engineered T-cells as a treatment option for solid tumours. One of the main challenges in the field of T-cell engineering is, for example, receptor specificity. Engineered T-cells endowed with high-affinity receptors proved significantly toxic when tumour-associated antigens were targeted that were also expressed, even at low level, on normal tissue [3,6–10], so-called 'on-target' toxicity. Other important aspects include choice of receptor format, strategies to prolong T-cell persistence and to reduce immunogenicity and sensitization of the suppressive tumour micro milieu [11].

We have designed a first-generation CAR directed against carboxy-anhydrase-IX (CAIX) and treated patients with CAIX-expressing metastatic renal cell carcinoma (mRCC) [12]. In this paper, we present a study overview and will

Key words: adoptive T-cell therapy, carboxy-anhydrase IX, chimeric antigen receptor, clinical study, immune monitoring, on-target toxicity, renal cell cancer.

Abbreviations: CAIX, carboxy-anhydrase-IX; CAR, chimeric antigen receptor; DLT, dose-limiting toxicity; GMP, good manufacturing procedure; IFN-γ, interferon-γ; IL-5, interleukin-5; mAb, monoclonal antibody; mRCC, metastatic renal cell carcinoma; MTD, maximum tolerable dose; TCR, T-cell receptor; TNF, tumour necrosis factor.

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summarize the clinical observations and immune monitoring performed on this clinical study including published and non-published data.

CAIX CAR T-cells

The first-generation CAR was constructed from the single chain variable domain (scFv) of the monoclonal antibody (mAb) G250 and the intracellular part of the γ-chain from the Fc(ε)RI receptor present on mast cells [13,14]. The G250 mAb recognizes an epitope on CAIX, which is frequently overexpressed on clear cell RCC [15]. Following retroviral introduction of the CAIX CAR into primary human T-cells, the CAR became surface expressed, which enabled T-cells to recognize CAIX and to exert antigen-specific effector functions, such as cytokine production and killing of RCC cell lines [16]. Next, we established and validated a good manufacturing procedure (GMP)-compliant protocol, based on T-cell activation with sCD3 mAb, retronectin-based transduction and IL-2 supported T-cell expansion to generate CAR T-cells in a closed culture system for patient treatment [16–18].

Pre-treatment observations – analyses of infusion products

The transduction protocol highly efficiently transduced all lymphocyte subsets, including CD4+, CD8+, CD57+ and TCR$\gamma\delta$+ T-cells [19]. Detailed phenotypic analysis showed that the CAR T-cell cultures were skewed towards differentiated CD8+ T-cells (as defined by markers CD45RA, CD45RO, CD62L, CCR7 and CD28). Of note, during culture all differentiation markers shifted along the anticipated T-cell differentiation lines [20]. At culture day 14, the majority of the T-cells were central memory (T_{CM}) and effector memory (T_{EM}) T-cells and the CAR expression was slightly higher on T_{CM} and T_{EM} when compared with the naive (T_N) and end stage (T_{ES}) T-cells [19]. Remarkably, all T-cell differentiation stages within both CD4+ and CD8+ T-cell subsets exerted similar levels of CAR specific CD107a mobilization, suggesting the CAR expression levels in all of these subsets was above a functional expression level and that in addition to CD8 CAR T-cells also CD4 CAR T-cells may exert effector T-cell functions [19].

Patients were treated with multiple T-cells infusions (see below) that were freshly prepared on culture days 14–18 in two treatment cycles. The CAR T-cell infusions of treatment day 1 (culture day 14) compared with day 5 (culture day 18) had similar T-cell phenotypes and proportions of CAR expressing T-cells, yet the expression level (mean fluorescence intensity, MFI) of the CAR was lower at day 5. The latter observation was confirmed by decreased CAR mRNA levels at treatment day 5 compared with day 1 although the CAR DNA levels were equal [21,22]. Our observations are in line with those of Burns et al. [23], who further showed that the loss of transgene expression in human lymphocytes transduced with a similar (MFG) retroviral vector was LTR-driven, and subject to global cellular mechanisms [23].

Further characteristics of the pre-infusion CAIX CAR T-cells are summarized elsewhere [24]. In short, T cells in the infusion products were 61 % CD8+ (median; range, 18–83 %) and 53 % (range, 24–65 %) expressed the CAIX CAR. The CAR T-cells had incorporated a median of 2.6 copies of the CAR transgene in their DNA (range, 1.2–12.9). We reported a median CAIX-specific cytolytic activity of 107 $LU_{20}/10^6$ CAR T-cells (range, 18–372) and interferon-γ (IFN-γ) production of 29 ng/24 h/10^6 CAR T-cells (range, 1–47). Specific IFN-γ production by T-cell from the therapeutic infusions was at least 20-fold higher than production of interleukin-5 (IL-5), tumour necrosis factor (TNF)-α and IL-4 [24].

Patient treatments

Between March 2003 and December 2010, we treated 12 patients with CAIX-expressing metastatic RCC, not amendable for curative surgery, and for whom no standard treatment existed [3]. Specific patient characteristics are described elsewhere [24].

Patients were not subjected to lympho-depleting pre-conditioning and were treated with two cycles of multiple intravenous (i.v.) infusions of CAR T-cells accompanied with subcutaneous (s.c.) IL-2 injections ($2\times$/d IL-2, 5×10^5 IU/m^2) during 10 days from the start of a treatment cycle. Patients were treated in three cohorts, i.e. featuring a treatment scheme that included adaptations in response to serious adverse events, see Figure 1 [24].

Cohort 1, the study was designed as a phase I/II study comprising of an in-patient dose escalation (i.e. day 1, 2×10^7 T-cells; day 2, 2×10^8 T-cells; day 3, 2×10^9 T-cells), followed by a consolidation dose of 2×10^9 T-cells at days 4–5 (treatment cycle 1) and 2×10^9 T-cells at days 17–19 (treatment cycle 2) (Figure 1 – scheme 1). In this scheme, three patients were treated, two of which developed dose-limiting toxicities in terms of liver enzyme disturbances, hence the treatment protocol was amended.

The amended protocol was a conventional 3×3 phase I study, applying a maximum of up to 10 CAR T-cell infusions at days 1–5 (treatment cycle 1) and days 29–33 (treatment cycle 2), at a start dose of 1×10^8 CAR T-cells per infusion and projected escalations to 2×10^8, 4×10^8, 8×10^8, 16×10^8, 20×10^8, 25×10^8 and 30×10^8 CAR T-cells per infusion. The study comprised two steps, the first without 'protective measures' in order to assess the net maximum tolerable dose (MTD) of the CAIX CAR T-cells with IL-2 support, and a second step including 'protective measures' being i.v. infusions of 5 mg of the anti-CAIX mAb G250 3 days before start of each treatment cycle. The pre-treatment with G250 mAb was intended to block CAIX in liver and prevent elevation in liver enzyme values, while not blocking CAIX in RCC metastasis [25–27]. In step 2, we aimed at increasing the MTD of the CAIX CAR T-cells and accomplish a clinical effective dose. In case dose-limiting toxicities were recorded in two patients at a particular dose level in step 1, subsequent patients would be treated at the same dose level in step 2.

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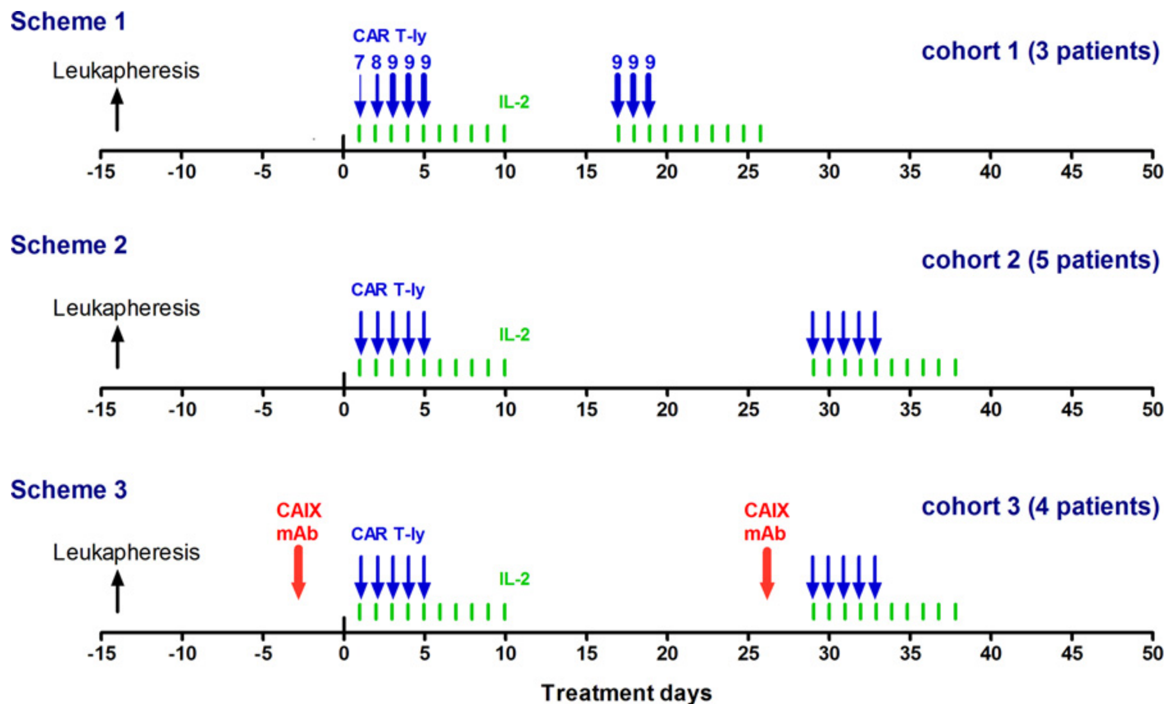
Figure 1 | **Treatment schemes**

Treatment schemes, adapted from: Lamers, C.H., Willemsen, R., van Elzakker, P., van Steenbergen-Langeveld, S., Broertjes, M., Oosterwijk-Wakka, J. et al. (2011) Immune responses to transgene and retroviral vector in patients treated with ex vivo-engineered T cells. Blood **117**, 72–82, as Supplemental Figure 1; © the American Society of Hematology. *In cohort 1 (scheme 1),* we treated patients with CAIX CAR T-cells in an in-patient dose escalation (i.e. day 1, 2x10^{7} T-cells; day 2, 2x10^{8} T-cells; day 3, 2x10^{9} T-cells), followed by a consolidation dose of 2x10^{9} T-cells at days 4–5 (treatment cycle 1) and 2x10^{9} T-cells at days 17–19 (treatment cycle 2), in combination with sub cutaneous (s.c.) injections of 5x10^{5} IU/m^{2} human recombinant IL-2 (Chiron, Amsterdam), twice daily administered at days 1–10 and days 17–26. For each treatment cycle a new T-cell culture was initiated from which fresh T-cell infusions were prepared. Patients 1 and 3 developed liver enzyme disturbances reaching CTC grades 3–4 following four T-cell infusions, which necessitated cessation of treatment in patients 1 and 3, corticosteroid treatment in patient 1 and reduction in the maximal T-cell dose to 2 x 10^{8} T-cells in patients 2 and 3 [3]. *In cohort 2 (scheme 2)*, we treated patients with CAR T-cells in a conventional phase I strategy with a maximum of 10 CAR T-cell infusions at days 1–5 and days 29–33 and staring at a CAR T-cells dose of 1x10^{8} per infusion and projected dose escalations to 2x10^{8}, 4x10^{8}, 8x10^{8}, 16x10^{8}, 20x10^{8}, 25x10^{8} and 30x10^{8} CAR T-cells per infusion. CAR T-cell infusions were in combination with IL-2, s.c., 5x10^{5} IU/m^{2} twice daily administered at days 1–10 and days 29–38. *In cohort 3 (scheme 3),* we treated patients as in cohort 2, but applied a strategy to block CAIX CAR recognition of cognate antigen on normal liver tissue. To that end we included an extra i.v. infusion of 5 mg cG250 mAb (kindly provided by L. Old, LIRC New York), 3 days prior to start of each series of CAR T-cell infusions, which blocks CAIX in the liver and leaving accessible CAIX at RCC tumour sites [26,27]. *Of note*, in this clinical trial patients were not subjected to pre-treatment lympho-depletion conditioning.



In cohort 2, five patients were treated according to step 1 of the conventional 3×3 phase I approach (Figure 1 – scheme 2). At the starting dose of 1×10^{8} CAR T-cells per infusion (maximum cumulative dose of 1×10^{9}), again a dose-limiting toxicity (DLT) was observed with respect to liver enzyme values in the third patient after 10 infusions (cumulative dose 1×10^{9} CAR T-cells) and in the fifth patient after three infusions (cumulative dose 0.3×10^{9} CAR T-cells). Therefore, cohort 2 was closed without a proper assessment of a MTD of CAIX CAR T-cells.

In cohort 3, four patients were treated as in cohort 2, but with pre-treatment of an i.v. infusion of 5 mg of the anti-CAIX mAb G250 3 days before start of each T-cell treatment cycle (Figure 1 – scheme 3). Three patients were treated at the starting (cumulative) dose of 1×10^{9} CAR T-cells without toxicity and the CAR T-cell dose was increased to 2×10^{9} CAR T-cells (cumulative) for the next three patients. Due to limited patient accrual because of competing treatments given the introduction of VEGFR-TKIs with proven activity in mRCC patients, the study was terminated after one patient in the second dose level of cohort 3.

Clinical observations

No clinical responses were noted and the median overall survival was 9.5 months (range: 3–33 months) for patients treated in cohorts 1 + 2 (n = 8 patients), and 12.5 months (6–24 months) for cohort 3 (n = 4 patients).

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Toxicities were restricted to elevations in blood levels of liver enzymes in four out of eight patients treated in cohort 1 and 2, as described above. Liver biopsies taken from three of these patients revealed CAIX expression on bile duct epithelium, discrete cholangitis with inflamed portal triangles and infiltration of T-cells, including CAIX CAR T-cells [3,24]. We concluded that the liver toxicity was probably due to the specific interaction of the CAR T-cells with CAIX expressed on the bile duct epithelium. Indeed, blocking of CAIX in the liver by G250 mAb infusion allowed treatment of the next four patients (cohort 3) at a (cumulative) dose of $1\text{–}2\times10^9$ CAR T-cells without any toxicity.

From one patient a peripheral metastasis was excised however no significant T-cell infiltrate was observed by immunohistochemistry.

Patient monitoring

CAR T-cells were quantified in blood by FCM using the anti-idiotype mAb NuH82. In addition, CAR DNA copies and CAR mRNA levels were quantified by qPCR and RT-qPCR, respectively. CAR T-cell, DNA and mRNA levels showed peak levels between treatment days 4–8 (for both cycles); peak levels were: CAR T-cells, median 2.7 cells/ul (range 0.8–10.0); CAR DNA levels; 0.070 fg/ul (0.018–0.566) which was equal to 10 transgene copies/ul (3–81); and CAR mRNA levels, 0.139 fg/ul (0.024–0.905). CAR T-cells, DNA and mRNA levels gradually decreased between both treatment cycles, but levelled off more rapidly after the second treatment cycle, see Figures 2(A)–2(C). This observation may have been caused by an anti-CAIX CAR immune response (see below), which became prominent after the second treatment cycle [28]. This notion is supported by the observation that patients treated in cohort 3, receiving the protective G250 mAb infusions, had detectable G250 mAb blood levels up to treatment day 10, but developed less anti-CAIX CAR immunity [28], and showed longer persistence of the CAIX CAR T-cells (Figures 3A and 3B).

Here we add additional data to our previous observation on the loss of CAR membrane expression following T-cell administration to patients [22]. The ratio of the CAIX CAR mRNA:DNA levels in blood gradually declined after treatment cycle 1 and showed a steep decrease after treatment cycle 2 (Figure 1D). Decreased CAR gene and surface expression are indicative for limited *in vivo* lymphocyte activation [23]. *In vivo* expansion of the infused T-cells was only seen in treatment cycle 1 in just three out of eight patients (cohort 2 + 3; Figure 2A).

Our observations on limited persistence of CAR T-cells concur with other reports on first-generation CARs [4,29,30]. However, in case virus specific T-cells were gene-modified with a first-generation CAR, these CAR T-cells persisted for months to years, likely due to repetitive viral re-stimulation [31]. Second generation CARs harbour intracellular co-signalling domains derived from molecules such as CD28 or 4-1BB. T-cells with second generation CARs showed improved *in vivo* expansion and prolonged persistence irrespective of lympho-depleting preconditioning [32–35]. These results have boosted the number of trials using second generation CD19 CAR T-cells to treat B-cell malignancies, which so far have shown impressive clinical results [36–38].

Blood cytokine levels were assessed using multiplex bead technologies, and revealed cytokine peak levels at day 5 (median; range, 5–8) in cycle 1 and at day 3 (range, 1–5) in cycle 2. Most prominent were elevations of IL-2, IL-5 and IFN-γ, potentially driven by IL-2 administration and *in vivo* activation of CAR T-cells [24]. Significant fluctuations were also recorded for: IL-1ra, IL-4, IL-12(p70), FGF-basic, G-CSF, GM-CSF, IP-10 and PDGF-bb. Analysis of cytokine levels after the last T-cell infusion revealed that IFN-γ and IL-6 levels correlated with numbers of peripheral CAR T-cells but not with liver enzyme toxicity scores [21].

Post-treatment PBMC displayed CAIX-specific T-cell functions, both cytolysis and IFN-γ production [24,39]. The CAIX-specific IFN-γ production peaked simultaneously (days 5–8) with the numbers of CAR T-cells in the circulation. Moreover, we showed that IFN-γ production by post-infusion PBMC correlated with pre-infusion CAIX CAR T-cell IFN-γ production potency [24]. Thus, CAR T-cells maintain their transgene-specific immune functions *in vivo*.

Immunogenicity

The CAIX CAR is constructed from the variable parts (Fv) of the murine mAb G250. The humanized (human/mouse chimeric) G250 mAb (cG250) has been applied for treatment of mRCC in monotherapy or in combination with IL-2 or IFN-γ. In such therapies, patients received weekly doses of 50 mg cG250 with a human Fc portion for 12 weeks, with minor induction of anti-G250 mAb immune responses [40].

However, patients treated with CAR T-cells developed distinct anti-CAIX CAR humoral immune responses in 7 out of 12 patients (not in three out of four patients treated in cohort 3) and cellular immune responses in nine out of ten evaluable patients [28]. Human anti-CAIX CAR antibodies were directed against the G250 mAb's idiotype and were able to neutralize CAR-mediated T-cell functions. Mapping of the anti-CAR cellular immune responses revealed reactivities against CDR2/3 and Vk FR3/4 domains. Remarkably, patients showed unique and single epitopes and none of the epitopes covered 'fusion' proteins that are part of the CAR. Of note, in this analysis we also detected immunity towards vector-encoded epitopes expressed by the CAR T-cells [28]. Thus, murine Fv domains in the context of a CAR and presented on T-cells can serve as strong immunogens [28,41] when compared with the soluble mAb [40]. Only a few studies report on immunogenicity of CAR or TCR modified T-cells [29,42], probably due to the commonly applied non-myoablative patient pre-treatment.

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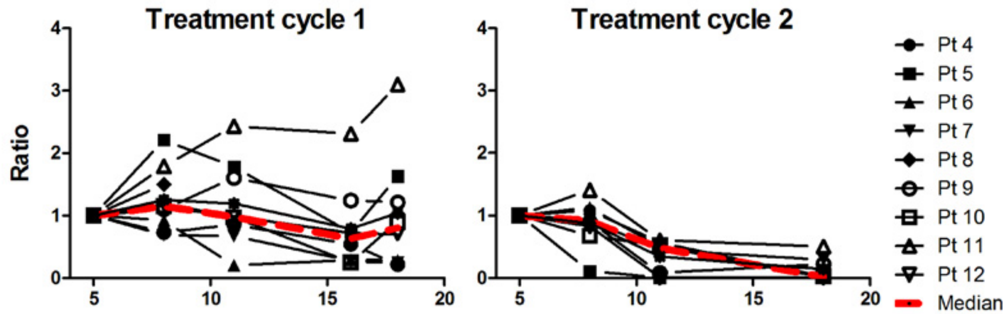
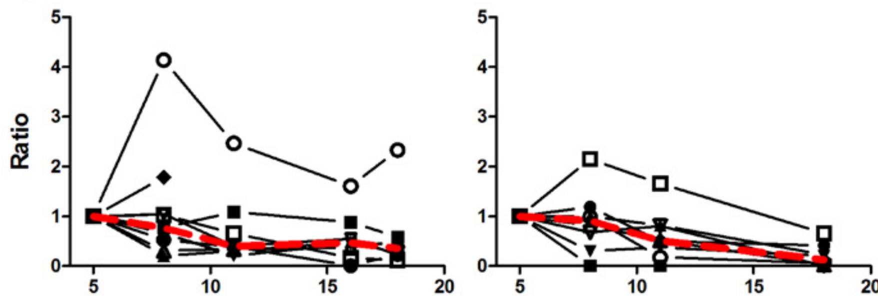
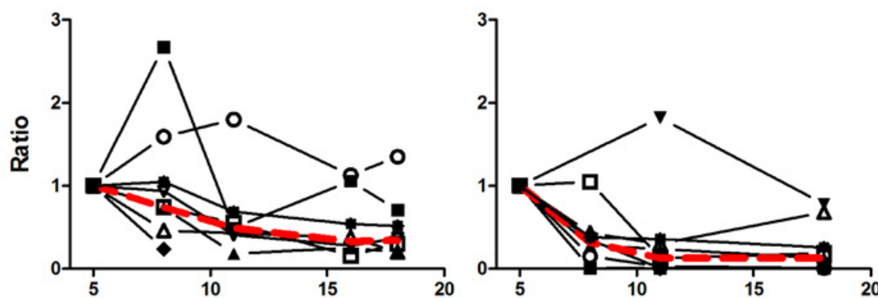
Figure 2 | *In vivo* **loss of CAIX CAR transgene expression**

Patients were treated with CAIX CAR T-cells (days 1–5) and monitored for blood numbers of CAR T-cells by FCM (**A**) and blood levels of CAR DNA (**B**) and RNA (**C**) by PCR and RT-PCR, respectively. Results are expressed relative to the value at treatment day 5 (day 5 ratio = 1). (**D**) Relative CAR RNA over CAR DNA ratio. Values for nine individual patients treated in cohorts 2 (filled symbols) and 3 (open symbols) and the median observation are shown. Of note, for patient 11 treatment cycle 1: in view of the decreasing RNA levels (C), the CAIX T-cell numbers (ratio; A) are relatively high; this observation might be accounted for by the relatively low CAIX T-cell measurement at day 5.







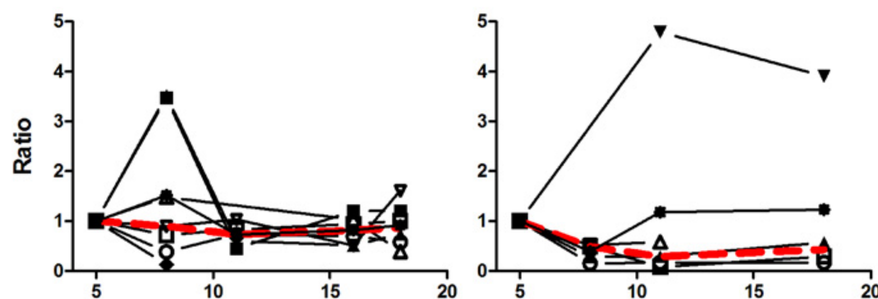


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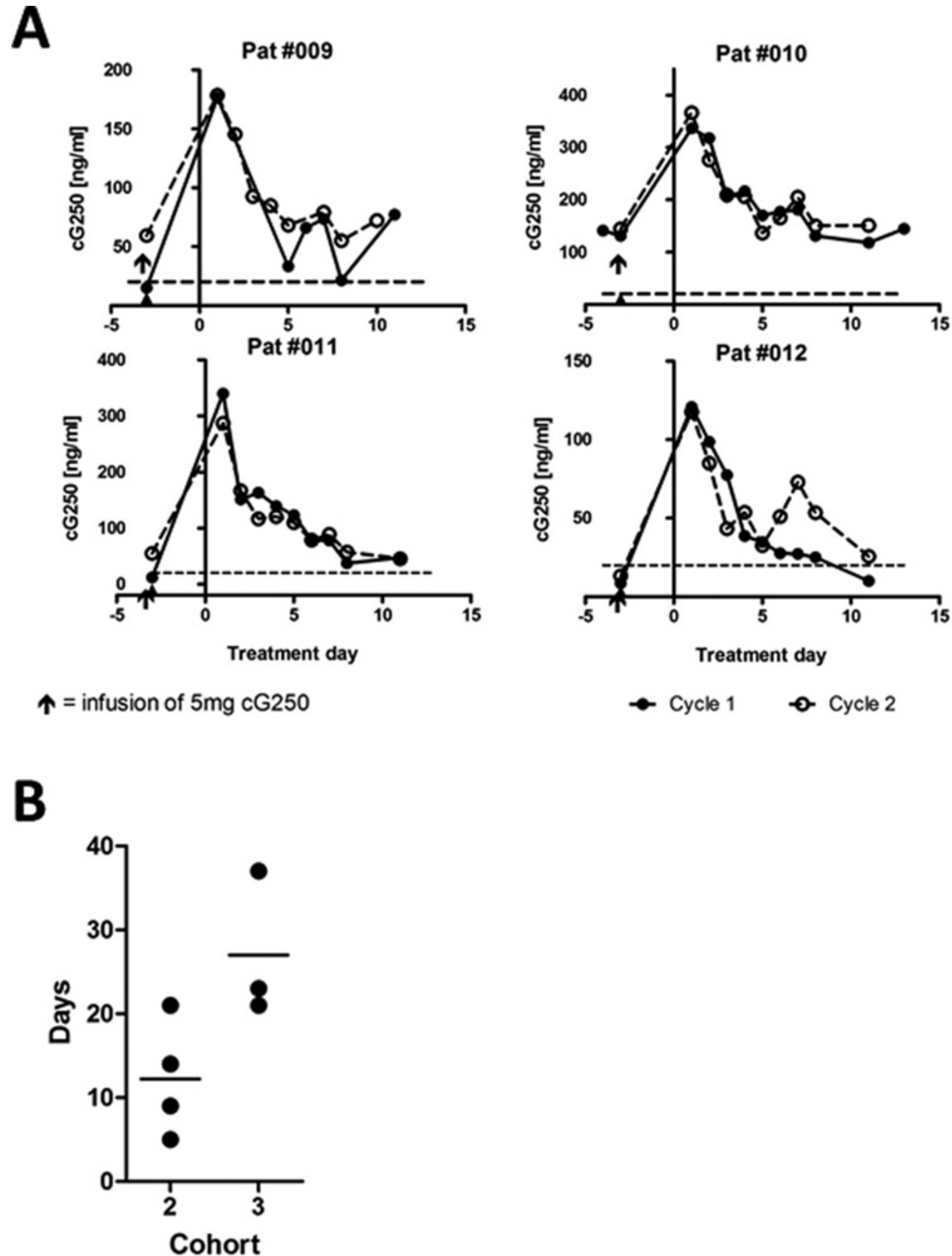
Figure 3 | Patient pre-treatment with CAIX mAb infusions prolonged CAR T-cell persistence

Patients in cohort 3 received i.v. 5 mg of the CAIX mAb cG250 (human/mouse chimeric G250 mAb), 3 days prior to T-cell infusion in both treatment cycles 1 and 2. Blood levels of cG250 were assayed by sandwich ELISA using the anti-Id G250 mAb NuH82. (**A**) Kinetics of cG250 blood levels during and after treatment cycles 1 and 2 for individual patients 9–12 (blood cG250 t1/2 values were pt 9: Cycle (C) 1, could not be determined; C2 (mean values) 2.3 days; pt 10, C1 2.9; C2 2.7 days; pt11 C1 1.7; C2 1.7 days; pt 12 C1 1.3, C2 1.5 days); (**B**) CAR T-cell persistence is defined as last day of detectable FCM values relative to the start of treatment cycle 2 in patients receiving a cumulative dose of $1x10^9$ CAIX CAR T-cells (cohort 2: pt 4–7; cohort 3: pt 9–11; *t* test, *P* value = 0.053). Of note, pt 12 received cumulative dose of $2x10^9$ CAIX CAR T-cells and developed anti-CAR antibodies from day 6 of treatment cycle II onwards [24], which interfered with the cG250 mAb detection assay.



Summary

Infusion product observations

- First-generation CAR genes showed homogeneous transduction efficiency and, once expressed, good antigen-specific function within different T-cell subsets/differentiation stages [18,19].
- Infused patient CAR T-cells displayed predominantly a central memory (TCM) and effector memory (TEM) phenotype [19].

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Clinical observations

- CAR T-cells did not significantly expand in vivo, nor persist >4 weeks post infusion and showed gradually decreasing CAR gene and surface expression [21,22,28].
- Blood cytokine profiles, in particular IFN-γ and IL-6, mirrored CAR T-cell presence and in vivo T-cell activity [21,24].
- CAR T-cells displayed antigen-specific functions [39].
- Patients presented with dose-limiting elevations of liver enzymes in blood highly likely as a consequence of specific recognition of CAIX on lining cells of the bile ducts by CAR T-cells [3,24].
- Blocking CAR by parental mAb (G250) infusion decreased liver enzyme values in blood [24].
- CAR T-cells induced both humoral and cellular immune responses in patients directed against murine Fv domains, and which preceded loss of CAR T-cells [28].

General remarks

- CAR T-cell treatment schedule, including two treatment cycles with repeated doses of freshly generated cells was logistically not convenient, and resulted in a reduced CAR expression in the latest T-cell infusions [21] and induction of anti-CAR immune responses [28].
- Study was stopped due to competing therapies with TKIs prior to establishing a therapeutic dose. No clinical responses were recorded.
- CAIX CAR T-cells displayed strong on-target effects, however the presence of CAIX on liver tissue rendered this treatment not feasible to explore further.

Recommendations

We recognized as major limitations in presented first-generation CAIX CAR T-cells clinical study, (i) the on target/off organ toxicity; (ii) immunogenicity of the CAIX CAR receptor (supported by treatment scheme) and (iii) lack of T-cell persistence and therapy efficiency.

In order to improve safety of receptor-engineered T-cell therapy, target antigens should be selected that are uniquely expressed by tumour cells and not on normal somatic tissues. For CARs it is quite a challenge to find such tumour-specific targets, whereas for TCRs promising candidates have been identified within the groups of both cancer testis (CT) antigens and neo-antigens [43,44], e.g. MAGE-C2 [45,46] and NY-ESO [47]. In case a self-antigen is targeted, the biological effect should also be taken into account and, where possible, preserved, e.g. the effect of B-cell aplasia following CD19 CAR T-cells treatment can be overcome by immunoglobulin infusions [48]. Targeting CT antigens might reveal unanticipated toxicities when applying affinity enhanced TCRs [7,49,50], emphasizing the need for thorough preclinical screening [51].

In the vast majority of clinical studies, immunogenicity of the receptor has not been recognized as a possible limitation, most likely due to the applied non-myoablative preconditioning of patients in most studies, single T-cell infusions and a recent dominance of CD19 CAR T-cell studies [52,53]. Yet, our study clearly demonstrated the immunogenicity of xenogeneic protein sequences presented by T-cells [41]. Therefore, for construction of CARs and TCRs we advocate the use of human CDRs.

To date, it has been shown that T-cell persistence and therapy efficiency improves from modification of the receptor design, in particular, by including a co-stimulatory domain in the receptor [52,53]. In addition, T-cells armoured with features that can adapt the immune-suppressive tumour microenvironment, e.g. by receptor-mediated local production of cytokines, chemokines or scFvs also bear therapeutic potential [11,54–56].

T-cells with a 'young' phenotype demonstrate improved persistence and therapy outcome [57,58]. Strategies to generate T-cells with a 'young' phenotype *in vitro* include, (i) T-cell activation using CD3 and CD28 co-activation [59–61], (ii) T-cell culture with common-γ cytokine (IL-7, IL-15, IL-21) support in culture [60–63] and (iii) (pre-)selection of T-cells subsets (e.g. CD62L selections) [64]. These strategies are included in an upcoming TCR T-cell adoptive therapy clinical trial at Erasmus MC to treat patients with MAGE-C2 positive tumours with co-stimulatory TCR T-cells [46,61,65].

There still remains a need for markers in adoptive T-cell therapy, whether related to pre-treatment infusion product or post-treatment blood measurements, that correlate with therapy effectiveness [52]. Extensive monitoring of these experimental studies is a prerequisite for obtaining a better understanding of the biology and mechanisms and will reveal tools and recommendations to improve the adoptive receptor-engineered T-cell approach.

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