Expert Opinion

Re-targeting T-cells against cancer by gene-transfer of tumor-reactive receptors

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Background: Adoptive transfer of T-lymphocytes is a promising treatment for a variety of malignancies, but is often not feasible due to difficulties in generating T-cells reactive with the targeted antigen from patients. To facilitate rapid generation of cells for therapy, T-cells can be programmed with genes encoding for an antigen-specific T-cell receptor (TCR) or chimeric receptors. *Objective*: To discuss the molecular design and selected pitfalls of TCR gene modified T-cells and T-cells expressing chimeric receptors, so called T-bodies. *Methods*: A selected review of the recent literature. *Conclusion*: Clinical trials report so far only limited efficacy of adoptively transferred genetically modified T-cells. However, the recent progress in engineering tumor-reactive T cells is providing a promising basis to further explore this treatment modality.

Keywords: clinical trial, genetic engineering, T-body, TCR gene transfer

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1. Introduction

The application of molecular technologies to identify proteins differentially expressed by transformed cells is providing large numbers of candidate antigens that can potentially be targeted to selectively eliminate tumor cells by cancer immunotherapy [1,2]. Efforts to vaccinate patients against such antigens have yielded some provocative results, but only a small subset of patients have demonstrated therapeutic responses, probably reflecting the many *in vivo* obstacles to generating potent responses to these proteins, particularly in patients with an established malignancy [3]. An alternative approach of isolating and expanding reactive T-cells *ex vivo* followed by adoptive transfer into the patient circumvents many of these *in vivo* obstacles. Although this adoptive therapy approach has demonstrated significant clinical promise [4], generating the large numbers of T-cells required for adoptive therapy of cancer patients is often not feasible. Molecular technologies have now provided a means to more broadly capture the therapeutic potential of this treatment strategy (**Figure 1**). Genes encoding the α and β chains of a T-cell receptor (TCR) can be isolated from a T-cell reactive to the antigen of interest and restricted to a defined HLA allele, inserted into a shuttle expression vector, and then introduced into large numbers of T-cells of individual patients sharing the restricting allele and the targeted protein [5,6]. Alternatively a T-body approach is pursued, which combines benefits of the high tumor specificity of antibodies and the effector function and the homing abilities of T-cells. The chimeric receptor recognition unit is derived from the variable chain of an antibody recognizing proteins ideally selectively expressed at the cell surface of a cancer cell and the intracellular domain is usually composed of signaling

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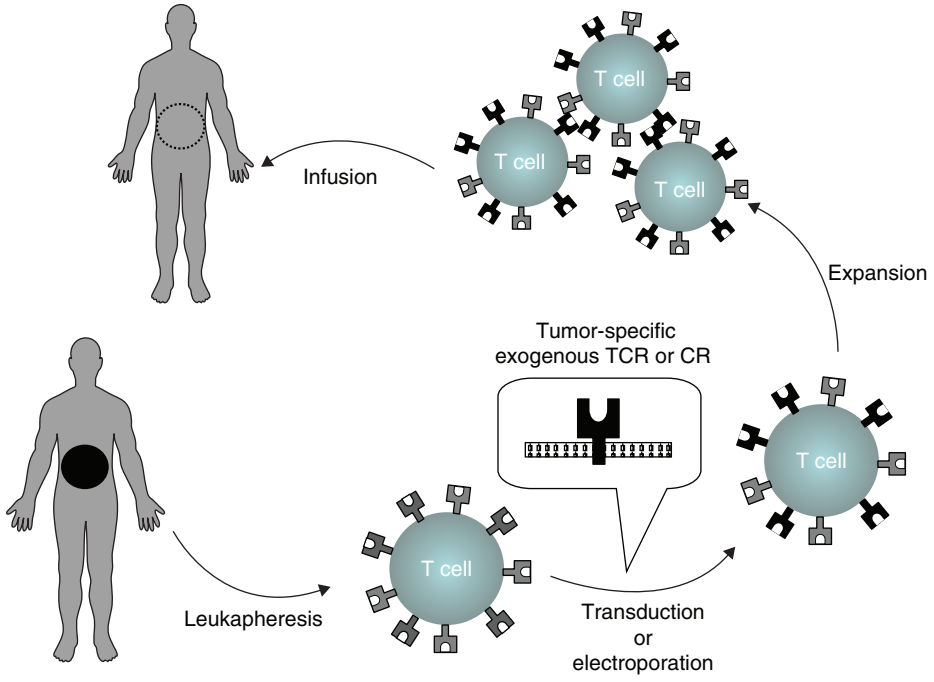

Figure 1. Adoptive transfer of genetically modified T cells. T cells isolated from a patient are genetically modified by, for example, retro-/lentiviral transduction or electroporation to express a tumor-specific receptor. After *in vitro* expansion cells are infused back into the patient.
CR: chimeric receptor; TCR: T cell receptor.

and co-signaling moieties of a T-cell receptor [7]. This review focuses on selected challenges and pitfalls of these strategies and published or ongoing clinical trials.

2. TCR gene transfer

Multiple virus- and tumor-reactive TCR-genes have already been successfully isolated and re-expressed in T-cells, including TCR-genes with specificity for HLA*0201 (HLA-A2)-restricted epitopes from melanoma antigens [8,9] and HLA-A2 and HLA*2402-restricted WT1-derived epitopes [5,10] However, to design broadly tumor-reactive TCRs for the next generation of clinical TCR gene therapy efforts, several aspects have to be taken into account. The TCR should target a protein that is widely expressed in tumors of any origin and cover a range of frequent HLA types. Furthermore, the targeted protein should be inherently involved in tumor transformation and selectively expressed by tumors and not by normal tissue. The TCR must be chosen for appropriate affinity for its ligand, be expressed strongly in T-cells after transfer and consequently allow reprogramming of $CD4^+$ helper/effector and $CD8^+$ effector T-cells. The design of introduced TCR chains must prevent pairing of introduced TCR chains with endogenous ones. This minimizes the generation of new potentially auto-reactive TCR specificities and simultaneously increases T-cell avidity by increasing the amount of functional TCR chains at the cell surface.

2.1 Engineering TCRs with high affinity against tumor antigens

A major challenge of current TCR gene transfer strategies is to define means to improve the avidity of T-cells transduced with a potentially tumor-reactive TCR, particularly in the context of the generally low affinity of available tumor-reactive TCRs. Therefore it is desirable to select tumor-reactive TCRs with the highest identifiable affinity [10-12] and explore strategies to increase TCR-affinity before transduction [13,14] or the (functional) avidity of the resulting TCR-transduced T-cells [5,15-17]. In this context, 'affinity' will be defined as the strength of binding of one receptor with its ligand, 'avidity' as strength of binding between multiple receptors and their ligands, and 'functional avidity' as the sensitivity of a T-cell response to a target cell expressing the relevant peptide-MHCs (pMHCs) [18].

Several approaches have been reported to select TCRs with highest affinity from the human T-cell repertoire. For example, alpha 3 domain mutants of peptide/MHC class I multimers have been used to allow the selective isolation of high avidity tumor-reactive $CD8^+$ T-cells [19]. To overcome the TCR affinity threshold determined by thymic selection, tumor-antigen-specific HLA-A*0201-restricted T-cells have been generated from HLA-A*0201 negative patients [11,20]. HLA-A*0201-transgenic mouse models [12] can, due to the differences in protein sequences in mice and men, provide TCRs with such a high affinity that these TCRs can also activate T-cells in the absence of the co-stimulatory CD8

molecule. However, expressing mouse TCRs in human T-cells might have a disadvantage as the expression of altered molecules results in a T-cell response against the transgene and rejection of transduced T-cells [21].

Different strategies have been examined to further increase the affinity of cloned TCRs used for transduction. TCR-phage [14] and -yeast [13] displays have been used as formats to express TCR chains and to then generate mutants that can be screened *ex vivo* for increased affinity. With these approaches, a library of TCR chains can be created generally following random mutation of defined regions such as CDR3 known to be important in recognition; the library expressed on the surface of the phage or yeast; the expressed mutated TCR-chains screened by tetramer-binding for increased affinity; and the cDNA encoding the highest affinity chains extracted, characterized and subsequently used for transduction. Consequently, these display strategies can be very effective, but are cumbersome and must be individualized for each TCR. Furthermore, not every TCR can be successfully expressed and modified by these technically challenging display strategies in non-mammalian systems, emphasizing the need for alternative techniques to increase TCR-affinity or functional avidity of a T-cell [18].

The functional avidity of a T-cell, as reflected by responsiveness to antigen, is modulated by T-cell surface *O*-or *N*-glycosylation [22,23]. Decreased glycosylation of surface proteins, has been reported to result in a decreased activation threshold. For example, decreased glycosylation of CD8 during thymic development is associated with increased affinity of CD8 with pMHC and subsequently improved signaling [24]. Moreover, a general deficiency in β1,6 *N*-acetylglucosaminyltransferase V (Mgat5) in mice, an enzyme in the *N*-glycosylation pathway, mediates increased T-cell activity *in vitro* and results in autoimmune disease *in vivo* [25]. Although deficiency of this *N*-glycosylation pathway enzyme in mice resulted in reduced *N*-glycosylation of all proteins, the reduced *N*-glycosylation of the TCRs appeared to specifically result in increased TCR mobility at the cell surface, enhanced recruitment to the synapse (TCR-clustering), improved TCR-engagement/downmodulation, and consequently enhanced functional avidity of T-cells. We demonstrated recently with several TCRs recognizing different antigens that the removal by point mutation of defined *N*-glycosylation motifs in the constant domains of TCR-chains can increase the functional avidity of T-cells transduced with these TCRs, and that this translates into enhanced recognition of tumor cells. Reducing *N*-glycosylation of TCRs might thereby directly enhance the interaction of TCRs with pMHCs and consequently increase T-cell activation [18]. As these *N*-glycosylation sites in the constant domain are conserved in all TCRs, this strategy should be easily translated to TCRs with any specificity.

An undesirable effect of an increased avidity of a tumor-antigen-specific T-cell might be the recognition of normal tissue either by aberrant recognition of self-antigens [26] or recognition of the specifically targeted tumor antigen, such as Wilms tumor 1 (WT1) [27], p53 [28], or murine double minute 2 homolog (MDM2) [11], due to the physiological expression of the protein at lower levels in normal tissues. It has indeed been reported that increasing the TCR affinity above a certain threshold can result in the recognition of self peptide and therefore auto-reactivity [26]. Therefore, high affinity TCRs need to be tested thoroughly for the 'recognition of self' [18].

2.2 TCR matching at the cell surface

Even after selecting a TCR with the highest affinity, the introduced exogenous α and β chains can potentially assemble as pairs not only with each other but with the endogenous TCR α and β chains, thereby reducing the number of appropriately matched exogenous αβ TCR-pairs at the cell surface and decreasing the achievable functional T-cell avidity (**Figure 2**). Such mismatched pairing poses a second substantive problem for clinically pursuing this strategy – the generation of novel αβ TCR-pairs of undefined and potentially self-reactive specificity, as these TCRs have not been subjected to the normal rigors of negative selection. Mismatched pairing of αβ TCR-chains has been clearly demonstrated to occur in TCR double-transgenic mice [29], in which all four of the expressed TCR-chains are known and can be followed. Since export of TCR-chains to the cell surface only occurs after formation of complementary α and β subunits [30], the potential for pairing of introduced TCR-chains with endogenous TCR-chains has also been demonstrated by introducing only single α or β TCR-chains into murine [31] and human [32] T-cells and detecting expression of the introduced chain on the cell surface. Unpublished data from different laboratories suggest that this can be indeed a substantial problem for adoptive immunotherapy.

To promote preferential pairing of introduced TCR-chains with each other, several strategies might be pursued (**Figure 2**). Recently, we [11,33] and others [15] have been using murine constant TCR-chain domains to enhance expression of human TCR-chains in human T-cells. However, murine constant domains might be immunogeneic in patients, thereby limiting the survival of transgene-transduced T-cells in patients [34]. The intracellular TCR-chain domains can also be linked to a signaling domain and this has been reported to result in CD3-independent signaling of TCR transduced T-cells [35]. However, as modulating the transmembrane domain might also result in unwanted effects such as an altered T-cell function [36] alternative approaches are being explored. Interactions between the extracellular domains, which can contribute to the formation of individual TCR-dimers and CD3-complexes [37,38], might be modified to facilitate inter-chain attraction. The interactions between exogenous domains of the TCR α and β chains are generally very weak [39], and the unique naturally occurring inter-chain disulfide-bond that occurs between the constant TCR α and β chains does not appear to contribute significantly to heterodimer stability [40]. However, disulfide-bonds engineered in other sites in the extracellular constant domain of the TCR αβ-heterodimer may have the potential

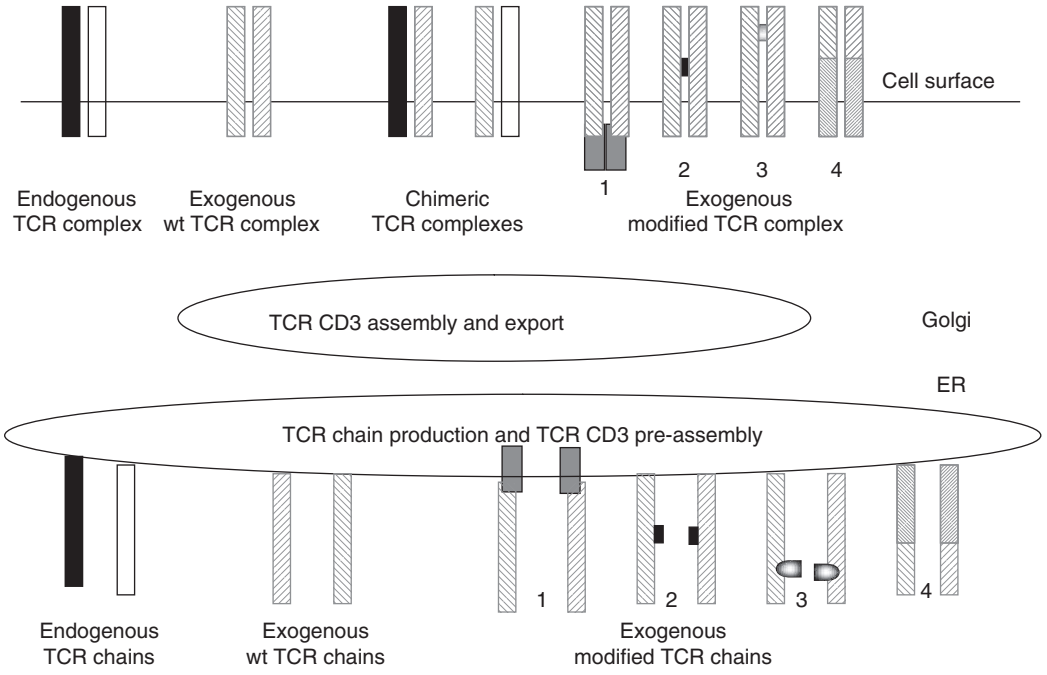

Figure 2. Improving matched TCR αβ chain assembly of introduced TCR chains. Introduced and endogenous α and β TCR chains can pair with each other. This creates TCRs of unwanted specificity and reduces the total number of correctly matched TCR chains at the cell surface. To promote pairing of the introduced chains genetic modifications can be made, such as 1 – addition of an intracellular signaling domain derived from CD3ζ; 2 – addition of cysteines or mutations to gain the 'hole-into-knob configuration' in the extracellular constant domains; 3 – selection of naturally occurring variable chains that preferentially pair with each other or generation of preferentially paired variable TCR chains by mutagenesis; and 4 – murinization of the constant αβ TCR chains.

to increase inter-chain affinity, as suggested by such disulfide-bonds enhancing formation of soluble TCR-heterodimers [41]. We found [5] and others confirmed [42] that such point mutations both promoted preferential pairing and increased expression of the introduced chains, resulting in greater avidity of the transduced primary CD8+ T-cells for WT1-expressing targets. Alternatively or additionally, a pair of amino acid residues in the crystal structure of a TCR that lie at the interface of associated TCR constant α and β domains can be mutated to invert the sense of this interaction analogous to a charged 'hole-into-knob' configuration [43]. Finally, also structural features of the variable TCR regions might be used to promote preferential pairing either by selecting TCRs with variable chains that pair preferentially to each other [44] or by introducing point mutations in the variable domain as reported for the expression of single-chain variable α and β fragments [45]. Although not formally tested so far, a combination of these different strategies might provide the best results in terms of preventing mismatched pairing.

2.3 Improving expansion and memory formation of TCR transduced T-cells

An antigen-specific T-cell expansion is one of the hallmarks for control of viral infections [46] and tumor growth [47]. Thus strategies that would allow to improve the expansion phase during an anti-tumor immune response would increase efficacy of immunotherapies. Therefore, the transgene should be incorporated into central memory T-cells in order to achieve a long term persistence of transduced T-cells [48]. This can for example be achieved by transducing and expanding genetically modified T-cells in the presence of cytokines such as IL7 and IL15 [49]. Detectable expansion of EBV-reactive T cells is found uniformly after T cell transfer into lymphopenic patients after hematopoetic stem cell transplantation [50], and consequently lymphodepletion has been successfully explored to improve T-cell expansion and clinical response rates following adoptive transfer of tumor-specific T-cells [47]. Elimination of $CD4^+CD25^+$ regulatory T-cells has been proposed as a key mechanism by which lymphodepletion augments adoptive T-cell-transfer-based immunotherapy [51]. A recent report suggested also that lymphodepletion removes endogenous cellular elements that act as sinks for cytokines capable of augmenting the activity of self/tumor-reactive $CD8^+$ T-cells [52]. Nevertheless, an expansion of tumor-antigen-specific T-cells has been observed after adoptive transfer only in some patients following lymphodepletion [4,53]. Thus, although current strategies help to increase T-cell expansion following adoptive T-cell transfer, improving the intrinsic capacity of a tumor-antigen-specific T-cell to expand more extensively following antigen-encounter would be further desirable.

An improved expansion of T-cells is a result of a reduced activation induced cell death (AICD) and/or an increased

cell division following antigen encounter. Two different TCR β chain alterations have been reported to impair Fas ligand (FasL) secretion while maintaining other effector functions, and thus should be candidates to reduce AICD and increase consequently T-cell expansion: A fusion of the transmembrane γ chain with a β chain ($\beta_{IIIchim}$) [54,55] and mutating the tyrosine$_{155}$ (Y_{155}) to leucine (L) in the trans-membrane domaine ($\beta_{Y155/L}$) [56,57]. However, a potential drawback of such mutation could be that the T-cell memory formation might be impaired after extensive expansion and indeed, a recent report suggests that alterations in this transmembrane region of the β TCR chain reduces the ability of a T-cell to build up a memory T-cell pool [36].

3. T-bodies

The T-body approach combines advantages of the high tumor specificity of antibodies and effector function and the homing abilities of T-cells by redirecting leukocytes with chimeric receptors whose recognition unit is derived from a variable chain of an antibody and the intracellular domain is composed from the signaling and co-signaling moiety of a TCR. Normally, a chimeric receptor is designed as a modular single chain molecule, with various structural and functional domains that allow to balance T-cell specificity and control the activation of a transduced T-cell (**Figure 3**) [7].

3.1 Specificity of T-bodies

The antibody-derived recognition unit is usually derived from the variable fragments (Fv) of the heavy and the light chains of a given antibody that are connected via a linker to form the single chain Fv (scFv). Such receptors allow the recognition of antigen in a MHC-independent manner and in the natural appearance of antigen at the cells surface. A wide variety of monoclonal antibodies against tumor-associated-antigens has been used to engineer T-bodies. For example, several groups used diverse antibodies against human EGF receptor 2 (HER2) [58-63], which is commonly overexpressed on breast-, prostate-, kidney-cancer, and many other tumors. Other popular antigens for T-bodies are B-cell markers, such as CD19 [64-66] and CD20 [67-69], which are associated with various B-cell-derived lymphomas. Optionally, using the modularity of the chimeric receptor, the recognition unit can get access to a ligand-binding domain of a heterologous receptor (linked to the TCR signaling moiety). One example is the extracellular domain of the CD4 molecule that binds gp120, an HIV envelope protein. Such T-bodies have been reported to be efficient against HIV-infected cells [70,71].

3.2 Modulating signaling in T-bodies

The scFv is linked through an extracellular linker to the transmembrane and cytoplasmic domains of lymphocyte triggering moieties such as the TCR/CD3 complex-associated ζ chain or Fc receptor γ chain [72-74]. Upon ligand binding, these domains signal via phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) and subsequently activate downstream kinases from the Src family. Alternatively, those down stream tyrosine kinases such as zeta-chain (TCR) associated protein kinase 70kDa (ZAP70) or spleen tyrosine kinase (Syk) can serve as a signaling moiety in the chimeric receptor and lead to an efficient and functional activation of T-cells [75]. Signaling by the chimeric receptor with one of these signaling domains occurs most probably via similar cascades as does endogenous TCR activation and results in cytolytic activity and secretion of cytokines like IL2, IFN-γ and TNF-α.

Adding a co-stimulatory signal might provide an advantage for optimal and persistent functioning of T-cells, their development into memory cells and re-activation, especially by targets lacking the ligands for co-stimulatory molecules such as tumor cells. For this matter, groups have designed tripartite chimeric receptors made of scFv linked to the intracellular part of co-stimulatory molecules such as CD28, OX40 (CD134), inducible costimulator (ICOS), programmed cell death protein 1 (PD-1) and 4-1BB (CD137) in series with the signaling moiety from the TCR and, thus, could provide both stimulatory and co-stimulatory signals for T-cell activation. Interaction of these different co-stimulatory receptors with their corresponding ligands results in a distinct function, depending on the nature of the stimulus and the antigenic history of a T-cell on which the chimeric receptor is expressed. For example, efficient CD28 signaling is accompanied by the induction of ICOS, which, in turn, co-stimulates $CD4^+$ T-cell activation [76]. Activation of OX40 promotes the expression of B cell leukaemia/lymphoma like X (*Bcl-XL*) and B cell leukaemia/lymphoma 2 (*Bcl-2*), enhances the number and survival of antigen-specific effector T-cells and generates potent antigen-specific $CD4^+$ T-cell memory [77]. Studies which compare these co-stimulatory molecules in the context of chimeric receptors showed notably enhanced cytokine release and killing when tripartite chimeric receptors were compared with chimeric receptors that did not contain any co-stimulatory signaling [78,79]. Finney *et al.* compared the effect of the co-stimulatory domains of CD28, ICOS, OX40 (CD134) and 4-1BB (CD137) in the context of the CD33-specific chimeric receptor in unstimulated human $CD4^+$ and $CD8^+$ T-cells. In this setting expression of domains derived from CD28 or ICOS were beneficial compared with domains derived from CD134 and CD137 in terms of mediating antigen-specific cytokine secretion and lysis of the target cells by T-bodies [78]. The advantage may be tumor- (costimulation) specific, since there appears to be no advantage to the CD134 domain when targeting e.g., B cell tumors which have costimulatory molecules. In another study, the CD137 signaling domain was used as the co-stimulatory moiety in the tripartite (anti-CD19 4-1BB-ζ) chimeric receptor and was found to elicit potent cytotoxicity of T-bodies against acute lymphoblastic leukemia (ALL) cells *in-vitro* [80]. Moreover, sequential addition of CD28 and CD134 together in the chimeric receptor specific to ganglioside GD2 gives an additional proliferative advantage of T-bodies in response to neuroblastoma cells [81].

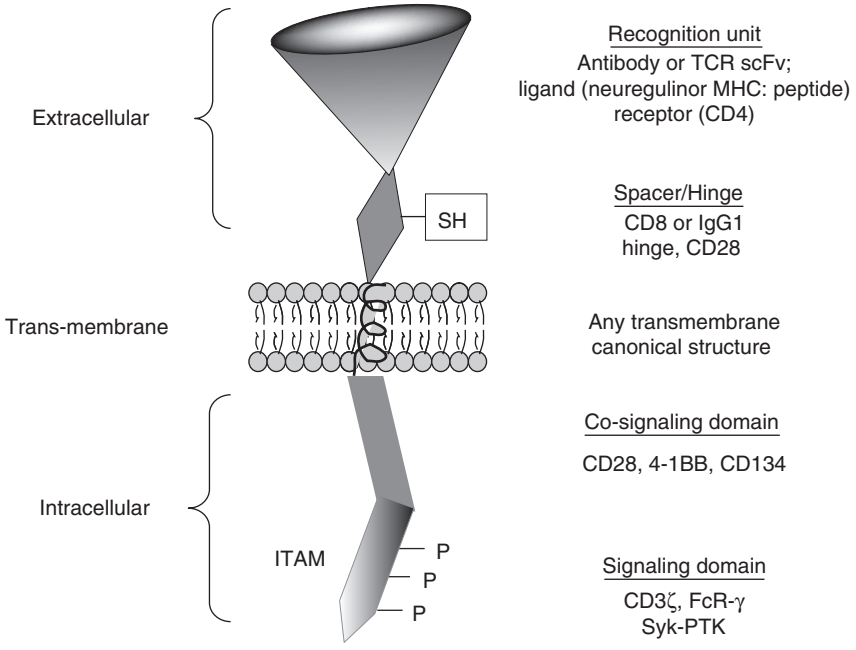

Figure 3. Chimeric receptor design. A schematic presentation of a modular chimeric receptor is shown. The extracellular domain is composed of the recognition unit that can be either scFv of the antibody or TCR or receptor such as CD4 and a hinge that keeps the recognition unit away from the membrane providing flexibility advantageous for recognition. Any canonical transmembrane structure can be used for the transmembrane domain, followed by co-signaling and signaling moieties.
ITAM: immunoreceptor tyrosine-based activation motif; PTK: protein tyrosine kinase; Syk: spleen tyrosine kinase.

Nevertheless, many groups have still chosen to selectively use CD28 as a costimulatory domain in chimeric receptors and showed that scFv-CD28-γ/ζ expressing T-cells efficiently kill tumors in different experimental settings *in vitro* as well as *in vivo* [74,82]. Moreover, Fridmann-Morvinski *et al* showed that expression of the tripartite chimeric receptor but not the CD28-truncated chimeric receptor could rescue activated T-cells from antigen-induced cell death [83]. In addition, recent findings showed that a CD28-domain-containing chimeric receptor enhances the resistance of the T-body to T regulatory cells [66].

Taken all together, the T-body approach demonstrates that antibody specificity, TCR signaling and co-signaling can be combined in one recombinant receptor, which can efficiently activate anti-tumor responses both *in vitro* and *in vivo*. However, it remains to be elucidated whether such an artificial signaling results in a physiological T-cell activation as minor changes in amino acid sequences in, for example, the TCR domain can alter memory T-cell formation [36]. Furthermore, these fusion proteins are potentially immunogenic [21].

4. Clinical trials

Table 1 summarizes most of the clinical trials using genetically modified T-cells in cancer patients that have been reported to date. Most of these trials were recently initiated and are still recruiting patients and therefore only some groups have published their observations so far. The main goal of these studies is to test feasibility and safety and to provide a 'proof-of-concept'. A completed Phase I study by Kershaw and colleagues [84] showed that adoptive administration of large numbers of T-bodies against α-folate receptor (FR) to ovarian cancer patients is safe, but these cells did not persist for a long time and did not localize to the tumor. Furthermore, half of the tested patients developed an inhibitory factor in their sera that significantly reduced reactivity of gene-modified T-cells to respond against FR-positive targets. This inhibitory activity was neutralized by protein G, suggesting that the inhibitor is most probably a human anti-mouse antibody. Another trial reported the development of human anti-mouse antibodies in patients with metastatic renal cell carcinoma that received infusions of T-bodies specific to carboxy-anhydrase-IX (CAIX) [85]. These results support the general idea that such anti-idiotypic responses can alter the therapeutic effect of genetically modified T-cells and strongly suggest considering the usage of human derived domains in the design of chimeric receptors. Though, the main problem of the last study was the development of grade 2 to 4 liver enzyme disturbances after four to five infusions of genetically modified T-cells. Liver biopsies from these patients revealed T-cells infiltrating around the bile ducts and CAIX expression on the bile duct epithelial cells, thus suggested an autoimmune-reaction of adoptively transferred T-cells. The trial was sustained and the protocol was modified to inject a low dose of a cG250 antibody to saturate liver tissue

Table 1. Summary of clinical trials.

Receptor type	Antigen	Type of cancer	Clinical outcome	Registration number on www.clinicaltrials.gov	PI and Ref.
Chimeric immunoreceptor IL-13 zetakine (with the Hy/TK selection/ suicide fusion protein)	IL13R α-2	High-grade malignant glioma		NCT00730613	Jensen MC
T-body	Carbonic anhydrase-IX	Renal cell carcinoma	Grade 2 – 4 liver enzyme disturbances Persistence of T-bodies up to 53 days Anti-idiotipic antibodies against scFv		Gratama JW [85]
T-body (with the Hy/TK selection/ suicide fusion protein)	CD19	Relapsed or refractory follicular non-Hodgkin's lymphoma		NCT00182650	Jensen MC
T-body scFv-CD28-CD3ζ	CEA	Colorectal cancer		NCT00673322	Junghans RP
T-body scFv-CD28-CD3ζ	CEA	Metastatic breast cancer		NCT00673829	Junghans RP
T-body	CEA	Adenocarcinoma		NCT00004178	Junghans RP
T-body scFv-CD28-CD3ζ	CEA	Gastric cancer		NCT00429078	Junghans RP
T-body MOv-scFv-CD3ζ	α-Folate receptor	Ovarian epithelial cancer	No toxicity, No tumor burden reduction, No long-term persistence	NCT00019136	Rosenberg SA [105]
T-body scFv-CD3ζ in ATC or EBV-specific T-cell + CD45 ab	GD2	Advanced neuroblastoma		NCT00609206	Louis C [89]
T-body CE7R sc-Fv-CD3ζ (with the Hy/TK selection/suicide fusion protein)	L1-cell- adhesion molecule (CD171)	Recurrent or refractory disseminated neuroblastoma	No toxicity, Short persistence (1 – 7 days) in patients with bulky disease, but significantly longer (42 days) in a patient with a limited disease burden.	NCT00006480	Park JR [86]
T-body scFv-CD3ζ	PSMA	Hormone-refractory prostate cancer		NCT00664196	Junghans RP
T-body: scFv-CD3ζ or scFv-CD28-CD3ζ	CD19	Low-grade B-cell on Hodgkin's lymphoma and chronic lymphocytic leukemia		NCT00608270	Brener MK and Heslop HE

ATC: Activated T-cell; CEA; Carcinoembryonic antigen; CR: Complete response; GD2: Ganglioside 2; EBV: Epstein–Barr virus; Hy/Tk; Hygromycin resistance/thymidine kinase; MART-1: Melanoma antigen recognized by T-cells; PR: Partial response; PSMA: Prostate-specific membrane antigen; SD: Stable disease.

Table 1. Summary of clinical trials (continued).

Receptor type	Antigen	Type of cancer	Clinical outcome	Registration number on www.clinicaltrials.gov	PI and Ref.
T-body: scFv-CD3ζ or scFv-CD28-CD3ζ in ATC or EBV-specific T-cell	CD19	B-cell non Hodgkin's lymphoma and chronic lymphocytic leukemia		NCT00709033	Brenner MK
T-body: scFv-CD3ζ or scFv-CD28-CD3ζ	CD19	B-cell non Hodgkin's lymphoma and chronic lymphocytic leukemia		NCT00586391	Brenner MK and Kamble R
T-body: scFvFc -CD3ζ	CD20	Mantle cell lymphoma or indolent B-cell non-Hodgkin lymphoma	2/7 CR 1/7 PR 4/7 SD	NCT00012207	Press OW [87]
T-body: scFvFc-CD28-CD137-CD3ζ	CD20	Mantle cell lymphoma or indolent B-cell non-Hodgkin lymphoma		NCT00621452	Till BG
T-body:	CD19	Chronic lymphocytic leukemia (CLL)		NCT00466531	Brentjens R and Riviere I
TCR	$gp100_{154-162}$	Metastatic melanoma		NCT00509496	Rosenberg SA
TCR anti-MART-1 F5	MART-1	High-risk melanoma		NCT00706992	Rosenberg SA [53]
TCR anti-MART-1 F5 + ALVAC-MART-1 vaccine	MART-1	Metastatic melanoma		NCT00612222	Rosenberg SA
TCR + p53 DC vaccine	p53	Metastatic cancer (with p53 over-expression)		NCT00704938	Rosenberg SA
TCR + ALVAC-CEA vaccine	$gp100_{154-162}$	Metastatic melanoma		NCT00610311	Rosenberg SA

ATC: Activated T-cell; CEA; Carcinoembryonic antigen; CR: Complete response; GD2: Ganglioside 2; EBV: Epstein–Barr virus; Hy/Tk; Hygromycin resistance/thymidine kinase; MART-1: Melanoma antigen recognized by T-cells; PR: Partial response; PSMA: Prostate-specific membrane antigen; SD: Stable disease.

and therefore to protect bile ducts from the damaging effects of scFv (G250)-positive T-cells. Even though, the liver toxicity was not beneficial for treated patients, these observations suggest that injected T-bodies are functional and can react against proteins expressed at the cell surface. Yet, it once again shows the importance of carefully choosing tumor-associated targets for T-cell-mediated immunotherapy and incorporating, for example, suicide genes into adoptively transferred transduced T-cells in order to dampen potential autoimmune responses [48]. In another study using a T-body specific to CD171, an adhesion molecule that is overexpressed in the metastatic neuroblastoma tumors in children, no treatment-associated toxicity was observed [86]. Persistence of genetically-modified T-cells was short in patients with bulky disease, but in patients with limited disease T-cells were detectable for a significantly longer time. However, only one patient experienced prolonged survival following the treatment. A 'proof-of-concept' trial in patients with relapsed or refractory indolent B cell non-Hodgkin's lymphoma and mantle cell lymphoma employed T-bodies against CD20 [87]. Also in this trial T-body administration was well tolerated and not toxic. In patients that received T-cell infusions together with subcutaneous IL2 for 14 days, T-bodies showed a prolonged persistence, compared with T-body administration without IL2. This is in line with the observation that persistence of genetically unmodified T-cell clones can also be improved by the application of IL2 [88] Moreover, six patients received cytoreductive treatment prior to adoptive transfer of T-cells that lead to complete clinical response in two patients and that response was preserved following T-body administration. These observations support the therapeutic potential of genetically modified T-cells for cancer therapy, yet, leaving a big window for improving clinical outcomes.

One possibility for improving anti-tumor activity is to introduce a tumor specific receptor into virus-specific T-cells as e.g., cytomegalovirus (CMV) or Epstein–Barr virus (EBV)-specific T-cells in order to improve long term persistence of adoptively transferred T-cells. This is based on the assumption that survival of genetically modified T-cells will be supported by triggering through the endogenous receptor, which is repetitively activated in order to control the CMV or EBV infection. A clinical trial performed in patients suffering from neuroblastoma compared the therapeutic activity of activated T-cells versus EBV-specific T-cells that were transduced with GD2-specific scFv-CD3ζ CR [89]. Indeed, EBV-specific T-bodies demonstrated superior persistence in patients when compared with T-bodies without virus specificity. Administration of such virus-specific T-bodies was safe and associated with tumor regression or necrosis in half of the patients tested. These results suggest that combination of tumor-specific receptors and endogenous virus-reactive TCRs improves survival of transferred T-cells and clinical outcome. However, whether the chronic antigen-stimulation can also result in a down-modulation of the introduced receptor [90] or whether chronic antigen-stimulation results in exhaustion or deletion of transferred T-cells [91] remains to be elucidated. However, the observation that gene-marked EBV-specific T cells have been detected for up to 38 months after infusion [92], suggests that at least EBV-specific T cells do not rapidly become deleted.

One clinical trial has been published so far that transferred TCR-gene-modified T-cells into patients [53]. This clinical proof-of-concept study demonstrates for the first time the safety and feasibility of administering large numbers of TCR-transduced T-cells to cancer patients. Using a strategy developed by this group to take advantage of the host providing homeostatic proliferative signals to transferred cells following lymphodepletion [47,93], the authors demonstrated remarkable long-term *in vivo* persistence of TCR-transduced T-cells as well as sporadic clinical responses. However, the response rate was well below the expected response rate anticipated from the administration of tumor infiltating lymphocytes (TILs) as reported by this group in similar situations [47,93] and there was no compelling correlation between early/late engraftment of TCR-transduced T-cells and the observed clinical responses.

5. Expert opinion

Although first clinical trials demonstrated the feasibility of the transfer of gene-modified T-cells and provide suggestive evidence for efficacy, multiple ongoing and new clinical trials are needed to improve this treatment option. This includes questioning the choice of antigen and the optimal design of the antigen-specific TCR or chimeric receptor in order to improve safety and efficacy of this treatment approach. The ultimate goal remains not only to treat an existing tumor burden but to implement a 'guardian against cancer', the tumor-specific memory T-cell, in order to prevent relapse. In this context which T-cell population should be transduced in order to guarantee memory T-cell formation and whether a large *ex vivo* expansion of transduced T-cells is necessary prior to application needs to be explored. Furthermore, persistence of genetically modified T-cells could be improved by vaccination strategies.

However, even then, αβ T-cell based immune-interventions are still hampered by the limited knowledge of antigens/proteins selectively expressed by cancer cells and in particular TCR based strategies are hampered by HLA-restrictions and tumor-escape mechanisms such as HLA-downmodulation, transporter associated with antigen presentation (TAP)-deficiency, or point mutations in the targeted epitope. T-bodies provide an alternative source of receptors to redirect a T-cell in a HLA-independent fashion against cancer cells but provide, due to the genetic engineering of fusion proteins, neo-antigens which can promote rejection of transduced T-cells. Furthermore, it is not clear whether a T-body is a functional fully competent T-cell. Therefore, our laboratory got interested in the ability of γδ T-cells and their receptors to mediate a broad tumor-reactivity. γδ T-cells have not been subjected to the normal rigors of negative selection. The recent elucidation of recognition of universal MHC-like stress-induced self-antigens such as

MHC class I polypeptide-related sequence A (MICA) [94], and mevalonate metabolites (phosphoantigens) [95] on tumor cells by γδ T-cells through the γδ TCR allows now a rational design of γδ T-cell or γδ TCR-based immunotherapies. The potential of γδ T-cells and their receptors to lyse tumor cells *in vitro* has been extensively demonstrated (for review see [96,97]). However an *in vivo* or *ex vivo* activation and expansion of cancer-reactive γδ T-cells in cancer patients is frequently not feasible as only a subset of patients harbors a fully cancer-reactive γδT-cell repertoire that can be expanded [98]. A similar situation has also been described for patients suffering from HIV or *Mycobacterium tuberculosis* infection [99]. Thus, the availability and capacity to activate and expand antigen-reactive γδ T-cells *in vivo* is essential for antigen-clearance but frequently impaired in diseases [98,99].

To overcome limitations of αβ and γδ T-cell based strategies, we propose to introduce defined cancer-reactive γδ TCR into αβ T-cells in order to redirect T-cells against cancer cells. It has been demonstrated that the transfer of an antigen-reactive γδ T-cell receptor into a Jurkat-cell line could transfer non-MHC restricted antigen-specificity [100,101]. Thus, the transfer of γδ TCR into αβ T-cells could be used for reprogramming non-tumor-reactive αβ T-cells from patients who are devoid of a suitable tumor-reactive T-cell repertoire. This strategy takes advantage of a universal-tumor reactive receptor from a γδ T-cell and the proliferation potential of αβ T-cells which is, in contrast to the proliferation capacity of γδ T-cells [98], still preserved in advanced diseases [53]. This concept would have further advantages: First, in contrast to αβ TCRs [5] γδ TCR do not pair with endogenous αβ TCR chains [102], thus this strategy prevents generation of unwanted specificities. Second, these genetically modified cells should be not immunogeneic as they do not harbor fusion or non-self-proteins as has been reported for multiple genetically modified T-cells [21]. Third, γδ T-cells and their receptors do not substantially mediate graft-versus-host disease (GvHD) [103] and fourth, the transfer of a γδ TCR into CMV-reactive T-cells or the transfer of a γδ TCR with shared reactivity against tumor and CMV infected cells would allow redirection of a T-cell against tumor cells and infection, which is appealing for patients following allogeneic stem cell transplantation [104]. However, it remains to be elucidated whether γδ TCR transduced αβ T-cells are immunologically fully competent T-cells in terms of effector, homing and memory function. The other major obstacle to this strategy is that ligands of γδ TCRs and the exact recognition mechanism need to be better defined.

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Declaration of interest

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