The T-Body Approach: Redirecting T Cells with Antibody Specificity

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Abstract "T-bodies" are genetically engineered T cells armed with chimeric receptors whose extracellular recognition unit is comprised of an antibody-derived recognition domain and whose intracellular region is derived from lymphocyte stimulating moiety(ies). The structure of the prototypic chimeric receptor, also known as a chimeric immune receptor, is modular, designed to accomodate various functional domains and thereby to enable choice of specificity and controlled activation of T cells. The preferred antibody-derived recognition unit is a single chain variable fragment (scFv) that combines the specificity and binding residues of both the heavy and light chain variable regions of a monoclonal antibody. The most common lymphocyte activation moieties include a T-cell costimulatory (e.g. CD28) domain in tandem with a T-cell triggering (e.g. CD3ζ) moiety. By arming effector lymphocytes (such as T cells and natural killer cells) with such chimeric receptors, the engineered cell is redirected with a predefined specificity to any desired target antigen, in a non-HLA restricted manner. Chimeric receptor (CR) constructs are introduced ex vivo into T cells from peripheral lymphocytes of a given patient using retroviral vectors. Following infusion of the resulting T-bodies back into the patient, they traffic, reach

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their target site, and upon interaction with their target cell or tissue, they undergo activation and perform their predefined effector function. Therapeutic targets for the T-body approach include cancer and HIV-infected cells, or autoimmune effector cells. To date, the most investigated area is cancer therapy. Here, the T-bodies are advantageous because their tumor recognition is not HLA-specific and, therefore, the same constructs can be used for a wide spectrum of patients and cancers. In addition, they can penetrate and reject not only vascular tumors but also bulky solid tumors. T-bodies have so far been prepared against a variety of tumors using scFv's derived from antibodies specific for tumor associated antigens. Proof of concept for the therapeutical benefit of cancer-specific T-bodies has been provided in animal models, and several phase I clinical trials are in process.

1 Background

The T-body approach, which combines antibody specificity with T cell stimulatory function within the context of a CR, was originally designed to better understand the mode of T-cell receptor (TCR) activation and the physicochemical parameters governing the T cell/antigen interaction (Gross and Eshhar 1992). Shortly thereafter, we and others realized the potential of this approach for cancer immunotherapy, combining the non-HLA restricted specificity of antitumor antibodies with the efficient tissue rejection of T cells (Eshhar et al. 1996).

The first configuration of an antibody-based chimeric receptor was composed of the two TCR chains. Here, the variable regions, Vα and Vβ, of the TCR chains were replaced with the VH and VL of the antibody light and heavy chains (Gross et al. 1989a, 1989b; Eshhar et al. 1996). Upon transfection of T-cell hybridomas and lines with the two chimeric chains (either CαVL and CβVH or CαVH and CβVL), a functional chimeric TCR/CD3 (chTCR) complex with antibody specificity was expressed on the cell surface. This chTCR associated with the CD3 complex and did not mix-assemble with the endogenous TCR chains. The double-chain configuration was instrumental for demonstrating that T cells can undergo activation in a non-MHC dependent or restricted manner (Gross and Eshhar 1992) and to highlight the role that TCR avidity and coreceptors (such as LFA-1, CD4, and CD8) play in T-cell activation (Lustgarten et al. 1991; Gorochov et al. 1993). The two-chain chTCR used in these early studies was directed at haptens and helped delineate the physicochemical parameters for TCR-mediated activation (Gross and Eshhar 1992). For these receptors, two genes were required to be co- or sequentially transferred into T cells to obtain a functional expression. Such double transfections were cumbersome for stable expression in naïve T cells. We therefore adapted the single chain Fv (scFv) unit to serve as an antibody recognition unit, and coupled it, through a hinge sequence, to either the CD3ζ or FCRγ activation chains (Eshhar et al. 1993). Such a single chain chimeric receptor was expressed as a homodimer or monovalent heterodimer associated with the endogenous CD3ζ chain and could, by itself, trigger T-cell activation and stimulate proliferation and effector functions including interleukin release and target cell killing upon antigen encounter (Eshhar et al. 1993).

This prototypic single chain configuration of the chimeric receptor (CR) has served as the template for today's T-body receptors. An additional significant development was the optimization of conditions to enable the transduction of naïve T cells, based on ex vivo activation and the use of retrovirus-mediated transduction (Rosenberg et al. 1990; Culver et al. 1991).

In this Chapter, I focus on the application of the T-body approach for cancer immunotherapy. Adoptive and passive immunotherapy have become a promising therapeutic option based on several clinical trials that demonstrated significant objective responses (and a few complete remissions) in cancer patients receiving tumor-specific T cells (Gomez et al. 2001; Dudley et al. 2002; Rosenberg et al. 2004; Bollard et al. 2004). Because tumor-specific T cells are rare for most human malignancies, the alternative of using genetically redirected, effector cells bearing ectopic receptors has become an attractive option for cancer therapy. Endowing T cells with antitumor specificity by the transduction of the two TCR chains has already proven effective in cancer patients (Morgan et al. 2006). This approach, unlike the T-body approach, is limited to individuals of certain HLA and is not applicable to tumors that escaped the immune system by failing to express surface MHC:peptide complexes.

2 Optimal Chimeric Receptor Composition

To serve as a cancer-specific therapeutic agent able to eliminate the large mass of cells in solid tumors, the T-body receptor must optimally discriminate the tumor from healthy tissue. Following its systemic administration to the patient, the T-body should migrate to the tumor site, interact with the tumor cell, undergo activation, and execute its effector function, culminating in cancer elimination. Optimal performance of this series of events is dependent on predefined, intrinsic properties of the transfected T cell that are triggered and regulated by the CR, and are dependent on its composition. The process of tumor rejection, similar to tissue rejection, is a complex one that requires both CD8 and CD4 cells that can mediate direct target cell killing and induce a local inflammatory response. Apparently, a single CR that interacts with its target antigen at high enough affinity can trigger the activation of both CD8 and CD4 T cells in which it is expressed. Nevertheless, for certain applications and specific targets, fine-tuning of the CR can be achieved by modifying several elements, mainly the activation and costimulatory sequences in its intracellular domain.

2.1 Combining Costimulatory and Stimulatory Signals

For optimal and sustained function of T cells, their development into memory cells and their reactivation, especially by targets lacking the ligands for costimulatory molecules (which are missing on many tumor cells), an added costimulatory signal is advantageous. It has been shown that CR that lack the capacity to provide

costimulatory signaling cannot activate resting or naïve lymphocytes, such as T cells derived from genetically modified stem-cells or from CR transgenic mice (Brocker et al. 1995). It is also well established that, in the absence of costimulatory signaling by CD28, resting T lymphocytes typically undergo anergy or apoptosis (Boussiotis et al. 1998).

These obstacles have been resolved by constructing CR in which the scFv is linked to the intracellular part of CD28 or other costimulatory molecules such as OX40 (CD134), CD40L, PD-1, or 4-1BB (CD137) (Finney et al. 1998; Finney et al. 2004). The effect of these costimulatory domains in the context of the CR was compared in unstimulated human CD4 and CD8 T cells and was found that cytokine release and killing activity in response to target cells was dramatically enhanced by all the costimulatory sequences relative to the CR that did not contain any costimulatory signaling moieties. No practical advantage was demonstrated by ICOS, OX40, or 4-1BB over the CD28-based tripartite CR. We designed a novel tripartite CR composed of an scFv recognition moiety, fused to the nonligand binding part of the extracellular and the entire transmembrane and intracellular domains of the CD28 costimulatory molecule, together with the intracellular domain of FcRγ (scFv-CD28-γ). Human PBL transducedwith such a CR gene demonstrate specific stimulation of IL-2 production and target cell killing (Eshhar et al. 2001). Many studies, from different groups, have demonstrated that out of the costimulatory domains, CD28 performed the best in various experimental settings (Gong et al. 1999; Hombach et al 2001; Haynes et al. 2002; Maher et al. 2002; Willemsen et al. 2005; Kowolik et al. 2006). Enhanced tumor rejection in mouse models using human and has been demonstrated (Pinthus et al. 2003, 2004; Westwood et al. 2005; Gade et al. 2005; Vera et al. 2006).To prove the ultimate requirement of CD28 for antigen-specific activation and development of mature naïve T cells, we have recently generated several lines of transgenic mice expressing CR under the control of T-cell-specific regulatory sequences. Unprimed, naïve T lymphocytes from mice transgenic for scFv-CD28-γ tripartite CR undergo high levels of proliferation, IL-2 secretion, and rescue from apoptosis following stimulation by plastic-bound cognate antigen (Friedmann-Morvinski et al. 2005). The rescue from apoptosis by CD28 in the context of T cells stimulation through the antigen-specific CR is an important factor in the persistence of the T-bodies in the patient where they are at the risk of antigen-induced cell death in the absence of B7 on the surface of the tumor target cell. Additional advantage is the recent finding that CD28 costimulation overcomes transforming growth factor (TGF)-β-mediated repression of proliferation of redirected human CD4+ and CD8+ T cells in an antitumor cell attack (Koehler et al. 2007). TGF-β is known for its immunosuppressive activity that is produced by regulatory T cells and some tumors. Along this line is the finding that the inclusion of CD28 to the CR enhances chimeric T-cell resistance to Treg (Loskog et al. 2006).

As to the use of 4-1BB signaling domain in the context of the CR, it was found to elicit potent cytotoxicity against acute lymphoblastic leukemia cells in vitro (Imai et al. 2004). Although the performance to date of CD28 appears quite satisfactory both in vitro and in vivo, the possibility of including additional or alternative moieties, or combinations of costimulatory domains should be further explored, especially to sustain and optimize the anticancer effect of T-bodies in vivo.

2.2 Signaling Domains

In contrast to the costimulatory moieties, only a few stimulatory domains have been used in the CR context. The original studies used both the CD3ζ and FcRγ subunits (Eshhar et al. 1993) and one group used the CD3ϖ domain (Schaft et al. 2006). All these domains signal through the immune T-cell activation motifs (ITAM) that contain a tyrosine, which undergoes phosphorylation as a result of the interaction of the TCR with antigen presenting cells. The phosphorylated ITAM facilitates docking of down stream kinases (such as ZAP70 and Syk) that are involved in signal transduction. FcRγ and CD3ζ contain a single ITAM, while CD3ζ contains three such motifs. No comprehensive comparison has been done so far to identify the most active domain in the context of the CR. We tend to prefer the FcRγ based on early studies showing that phosphorylation of the first ITAM of the CD3ζ leads to anergy (Kersh et al. 1999). Despite the lack of consensus, we have tried to bypass signaling through the ITAM that is impaired in T lymphocytes of tumor-bearing subjects (Mizoguchi et al. 1992). In a series of studies, we found that using the Syk cytoplasmic phosphotyrosine kinase as the signaling domain of the CR instead of an ITAM-containing signaling chain can efficiently induce T-cell activation (Fitzer Attas et al. 1998).

3 Preclinical Proof of Concept in Experimental Models

To date, many antibodies recognizing various tumor antigens have been used to generate CR that target T-bodies to variety of human tumors (for recent reviews see Kershaw et al. 2005; Friedman-Morvinski and Eshhar 2006). In the first preclinical trial (Moritz et al. 1994) of T-body-mediated therapy, mouse T cells expressing HER2-specific CR were injected subcutaneously (s.c.) into nude mice together with a target tumor transfected with the human erbB2 gene. In this model, it was observed that tumor development was significantly delayed. In a more recent study by the same group (Altenschmidt et al. 1997), it was shown that HER2-specific splenic T cells repeatedly administered directly into erbB2-expressing mouse mammary tumors result in total tumor regression. Using murine CTL expressing CEA-specific CR (Haynes et al. 2002), the group in Melbourne showed an in vivo effect against colon carcinoma. The CTL-mediated effect in this study required perforin and IFNγ and was independent of FAS-L or TNF. A more clinically relevant experiment used murine tumor infiltrating lymphocytes transduced with a folate binding protein (FBP)-specific CR (Hwu et al. 1995). The tumor target was a syngeneic metastatic sarcoma, transduced with the human FBP gene. When injected intravenously into mice, together with daily injections of IL-2, FBP-specific T cells induced a significant reduction in the number of lung metastasis.

To test whether human T-bodies (usually from peripheral blood lymphocytes (PBL) derived by retroviral vector transduction) could specifically recognize and eliminate human tumor cells or xenografts, most models used immune deficient

SCID or nude mice. In such a system, we demonstrated the ability of HER/2-specific human T-bodies to reject large established (subcutaneous and orthotopic) prostate cancer human xenografts (Pinthus et al. 2003) and tumors derived from human breast cancer cell lines (Morvinski-Friedman and Eshhar, unpublished). It should be emphasized that in these studies, the T-bodies were injected directly into the tumor; such an administration route is a valid option for primary solid tumors that are accessible. A simpler and more desirable route of effector cell administration is systemic infusion. Here, the redirected T-bodies must circulate in the body and migrate to their tumor target, whether a localized primary tumor, secondary metastases, or disseminated residual tumor cells following conventional therapy. Using systemic administration, most published (and many unpublished) studies showed only a mild and transient effect. On the basis of the experience of Rosenberg's group using either human tumor infiltrating lymphocytes (TIL) for melanoma treatment (Dudley et al. 2002) and results obtained in the murine Pmel system by Restifo and Rosenberg (for recent review see Gattinoni et al. 2006), it was found that treating patients (or mice) with mild lymphodepletion agents (such as cyclophosphamide and fludarabin alone, or together with sublethal irradiation) to create a lymphopaenic environment before T-cell transfer, dramatically improves the antitumor response of the adoptively transferrd cells in the recipient. Indeed, when we treated recipient SCID mice harboring a prostate cancer bone lesion in a similar way, the T-bodies resulted in considerable antitumor respones including complete cure in a significant number of mice (Pinthus et al. 2004). Several mechanisms have been suggested for the effect of lymphodepletion (for reviews see Klebanoff et al. 2005; Wrzesinski et al. 2005) including overcoming homeostatic control, eliminating regulatory cells and cytokine sink (Gattinoni et al. 2005). Because our studies were done in both lympho and myelo deficient strains of SCID mice, in which the homeostatic control and suppressive effects of lymphodepletion do not play a role, we found that at least part of the benefit of lymphoablative preconditioning is due to an increase in SDF-1, which is induced by the regenerative process following the preconditioning treatment. Apparently, the elevation of SDF-1 compensates for the low expression of its receptor, CXCR4, on the ex vivo manipulated cells (Pinthus et al. 2004).

Studies using adoptive cell transfer for the treatment of cancer have been greatly intensified in recent years, mainly due to the lack of success of therapeutic cancer vaccination using active immunization (Rosenberg et al. 2004); these trials have provided many lessons that could be adapted to the T-body approach. Yet, adoptive T-body transfer requiring ex vivo manipulation to introduce the CR genes suffers from the fact that the T cells must be activated to enable the gene transfer process. Such activation, usually induced by anti-CD3 and CD28 antibodies, drives the cells to differentiate into effector cells with modified expression of surface receptors and adhesion molecules, thus resulting in impaired migration patterns and limited persistence in the recipient. Much effort has been invested in recent years in order to improve these key issues that directly affect the therapeutic potency and potential of the T-bodies. Approaches that are currently being tested include the use of nonretroviral vehicles, such as transfection of CTL with naked DNA (Jensen et al. 2000) and RNA-based transfection (Johnson et al. 2006). In addition, cytokines such as IL-2,

IL-7, IL-15, and IL-21 are being tested to support the homeostatic proliferation of T cells in the recipient (Brentjens et al. 2003; Klebanoff et al. 2004; Wang et al. 2005; Hsu et al. 2005; Hwang et al. 2006; Hsu et al. 2007). These interleukins are added during the ex vivo preparation phase and/or as part of the in vivo treatment. One notable example is IL-21, which directs cells to differentiate in vivo into central immune memory cells, rather than into the short-lived effector memory phenotype (Klebanoff et al. 2006). Other variables that are being studied in order to improve and optimize T-body function in vivo are selecting the best costimulatory moiety (as discussed above), not only elimination of regulatory cells from the cancer patient as described above, but also their removal from the lymphocyte population set aside for transduction. Finally, to provide the T-body with growth signals in vivo that will prolong their persistence regardless of the CR signals, several groups have introduced the CR to T cell populations already specific to a common viral antigen (such a EBV (Rossig et al. 2002) or Flu (Cooper et al. 2005)).

A different approach to avoid the need for ex vivo manipulation of T cells is to introduce the CR into hematopoietic stem cells that will eventually differentiate and mature into T cells. Wang et al. (1998) evaluated the potency of murine bone-marrow stem cells, transduced with FBP-specific CR gene, and found that the growth of an FBP-expressing tumor was retarded. Interestingly, T cells are not directly involved in this process, as depletion of CD4 and CD8 cells does not diminish the antitumor activity. It was therefore suggested that NK cells and/or macrophages expressing the anti-FBP CR are responsible for this antitumor effect. More recently, Baltimore's group described a method to genetically program mouse hematopoietic stem cells (HSC) to develop into functional CD8 or CD4 T cells of defined specificity in vivo (Yang et al. 2002 and 2005). To this end, they engineered a bicistronic retroviral vector that efficiently delivers genes for both α and β TCR chains of the TCR to the HSC. By combining cells modified with CD8- and CD4-specific TCRs and boosting with dendritic cells pulsed with cognate peptides, complete suppression of tumor growth was achieved, and even established tumors regress and are eliminated following dendritic cell/peptide immunization. These experiments highlight an extension of the T-body approach through the administration of effector cells into patients undergoing systemic cyto-ablative therapy. Thus, cancer patients treated with bone marrow transplants could be reconstituted with genetically altered stem cells. Immediate possible candidates for such a treatment are leukemic patients receiving stem cell grafts. The tripartite CR is the construct of choice for such treatment, as its built-in costimulatory signaling function supports the priming and activation of naïve T cells to mature specific effector activity.

4 Clinical Trials

As shown in Table 1, several phase-I clinical trials using T-bodies for cancer therapy have been initiated, and several others are on-going or in various stages of planning. Because results from only a few of these studies have been published to date,

Table 1 T-Bodies in clinical trials

Tumor	Antigen	Group	Status[a]
Ovarian	FBP	Hwu, Rosenberg, NCI	Performed
Colorectal ca.	TAG-72	McArthur, Cell Genesys	Performed
Colorectal & breast ca.	CEA	Junghans, Harvard	Performed
Renal ca.	Carboxyanhydrase IX	Gratama, Rotterdam	Ongoing
Neuroblastoma	CD171	Jensen Seattle/City of Hope	Ongoing
Glioblastoma	IL-13 Receptor[b]	Jensen, City of Hope	Ongoing
Neuroblastoma	G(D)2	Brenner, Baylor College of Med.	Ongoing
Gastric ca.	CEA (2nd generation)	Junghans, Roger Williams	Recruiting
Prostate ca.	PSMA	Junghans, Roger Williams	Recruiting
Leukemia	CD19	Jensen, City of Hope	Recruiting
Leukemia	CD19	Hawkins, Manchester	Recruiting
Leukemia	CD19	Sadelain, Sloan Kettering	Recruiting
Leukemia	CD19	Brenner, Baylor College of Med.	Approved
Leukemia	CD19	June, Univ. Pennsylvania	Pending
Pancreatic ca.	Mesothelin	June, Univ. Pennsylvania	Pending
Colorectal ca.	CEA	Hawkins, Manchester	In planning
Prostate ca.	PSMA	Sadelain, Sloan Kettering	In planning
Myeloma	Lewis-Y	Kershaw, Melbourne	In planning
Cutaneous lymphoma	CD30	Abken, Cologne	In planning
Lymphoma	CD20	Cooper, MD Anderson	In planning

[a] Updated to April 2007
[b] Redirected by IL-13 ζ CR (not antibody based)

much of the information in this section is derived from registries and from personal communications. One fully documented phase I trial was conducted in HIV infected subjects receiving autologous lymphocytes bearingthe CD4-ζ CR (Mitsuyasu et al. 2000). About half of the patients also received concurrent IL-2 infusions for five days. The treatment was well tolerated with grade 3 or 4 adverse events predominantly associated with the IL-2 infusion. In some patients, a transient decrease of the viral load was observed in the plasma and the rectal mucosa, the tissue reservoir for HIV. All 24 subjects tested negative for replication-competent retrovirus for up to one year after cell infusion. Cell Genesys, which carried out this study, also conducted phase I clinical trials in colorectal patients using the anti-TAG72-ζ CR made from the humanized CC49 mAb (Warren et al.1998). This trial, however, was terminated due to the identification of anti-idiotypic antibodies in the patient sera, which caused difficulty in interpretation of the results.

The group of Junghans tested 24 doses of CEA-specific CR-bearing lymphocytes, with a total dose of up to 10^{11} cells per patient. The treatment was reported to be adequately tolerated, with only two minor adverse effects observed in two colorectal carcinoma patients (Junghans et al. 2000). Hwu and colleagues (Kershaw et al. 2006) at the NCI conducted a phase I clinical trial in ovarian cancer patients using T-bodies expressing a CR that we generated against human α folate receptor, also known as FBP. This trial demonstrated that large numbers of gene-modified

tumor-reactive T cells can be safely given to patients, but these cells do not persist in large numbers in the long term. No reduction in tumor burden was seen in any patient. Tracking ^{111}In-labeled adoptively transferred T cells revealed that the T cells did not localize to the tumor, except in one patient where some signal was detected in a peritoneal deposit. PCR analysis showed that gene-modified T cells were present in the circulation in large numbers for the first two days after transfer, but these quickly declined and became barely detectable one month later in most patients. Five out of eight patients who received a dose escalation of T cells in combination with high-dose IL-2 experienced some grade 3 to 4 treatment-related toxicity that was probably due to IL-2 administration, which could be managed using standard measures. Patients in cohort 2 who received T cells with dual specificity (reactive with both FR and allogeneic cells), followed by immunization with allogeneic peripheral blood mononuclear cells, experienced relatively mild side effects with grade 1 to 2 symptoms. Neutralizing antibodies were found in some of the patient sera, specific to the murine anti-FBP MoV18 mAb.

A group at Daniel den Hoed Cancer Center in Rotterdam reported a phase I clinical trial in renal cell cancer (RCC), using autologous T lymphocytes modified with a CR specific for carboxy anhydrase IX (Lamers et al. 2004, 2006). Infusions of the modified T lymphocytes were initially well tolerated. However, after four to five infusions, all three patients began to develop liver enzyme abnormalities. This was explained by the reactivity of the genetically modified cells with low levels of carboxy anhydrase IX expressed on the bile duct epithelium, limiting treatment to only low doses of CR-expressing T-bodies. The results of this study showed that the T-bodies exert CR-directed functions in vivo. Several patients in the trial also developed antibodies to the murine G250 scFv. Because of these side effects, this trial was put on hold and awaits renewal pending the application of systemic anti-carboxy anhydrase IX antibodies to block antigen expression on the bile duct. The results of a safety/feasibility trial using human CTL clones redirected at metastatic neuroblastoma was recently published (Park et al. 2007). In this trial CD8+ CTL clones were transfected with anti-CD171 CR and the selection suicide expression enzyme HyTK. Six children with recurrent/refractory neuroblastoma received 12 infusions. No overt toxicities to tissues known to express the CD171 adhesion molecule were observed. The persistence of the modified CTL in the circulation was short (1–7 days) in patients with bulky disease, but significantly longer (42 days) in a patient with limited disease burden. The authors suggest this pilot study set the stage for clinical trial in the context of minimal residual disease.

Table 1 also includes clinical trials that are either ongoing or in advanced phases of preparation (e.g., awaiting authorization from regulatory bodies). Many of these trials have been designed based on lessons learned from the preclinical animal models, e.g., use of humanized scFv, inclusion of the CD28 costimulatory domain, use of lentiviral vectors to transduce the PBL, inclusion of homeostatic interleukins in the ex vivo procedures used to prepare the T-bodies, transfection of both CD4 and CD8 T cells, use of T-bodies made from autologous T cells that are also specific to viral antigens such as EBV and influenza. Very importantly, in several of these studies, lymphoablative pretreatments will be used to precondition the patients before the

administration of T-bodies. Most of the trials will target blood borne tumors such as lymphoma or leukemia, using anti-CD19 or CD20 scFvs from humanized antibodies. Although less challenging than solid tumors, it is hoped that the results of using T-bodies against these targets will demonstrate some dose-dependent effect, by decreasing the tumor load, and thereby paving the way for applying the T-body approach against more challenging solid tumors. Introduction of genes to T cells using retroviral vectors has been proved safe and it is clear today that the risk of leukemia that occurred in patients receiving retroviral vector-mediated gene transfer into HSC does not exist for mature T cells. Potential severe side effects seen in some patient in the clinical trials reported above are manageable. A careful selection of the antibody whose scFv will serve to redirect the T-bodies, both in terms of specificity and affinity, will diminish the risk of damaging essential healthy tissues and side effects of IL-2 could be controlled and hopefully will be prevented when the persistence of the T-bodies in the body will be improved as discussed above.

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