Immunology Letters 211 (2019) 13–22

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Review

The UniCAR system: A modular CAR T cell approach to improve the safety of CAR T cells

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ARTICLE INFO

Dedicated to my mentor and friend: Morris Reichlin † 2018.

Keywords:
Immunotherapy
Chimeric antigen receptor
T cells
UniCAR
BiTE
Bispecific antibody

ABSTRACT

The idea to eliminate tumor cells via our own immune system is more than a hundred years old. However, a real break through came first with the development of check point inhibitors, bispecific antibodies (bsAbs) and T cells genetically modified to express Chimeric Antigen Receptors (CARs). Eventhough the clinical application of T cells equipped with CARs can lead to a complete remission, unfortunately, their application can also cause severe or even life threatening side effects as their activity can no more be adjusted once given to the patient. For targeting of tumor cells expressing tumor associated antigens (TAAs) which are not limited to tumor cells but also accessible on healthy tissues CAR T cells should not be permanently in a killing mode but be equipped with some kind of a switch whereby the activity of CAR T cells can reversely be turned "on and off ". Moreover, in case of cytokine release syndrome (CRS), tumor lysis syndrome (TLS), or other deadly side effects the possibility of an emergency shut down of the CAR T cell activity should exist. Modular CAR variants such as the UniCAR system may fulfill these requirements.

1. Introduction

Already in 1989 the group around Z. Eshar described T cells genetically modified to express artifical receptors [1]. Originally termed as T-bodies, they are nowadays better known as Chimeric Antigen Receptors (CARs) [e.g. 2–5]. CARs are synthetic type I receptors usually consisting of (i) an extracellular target recognition domain, (ii) a transmembrane domain, and (iii) an intracellular domain containing a signalling motif allowing to activate T cells [e.g. 3,4,5, see also Fig. 1]. CAR T cells can target surface antigens independently of MHC expression. The extracellular domain of a CAR usually is a single chain fragment variable (scFv) which can be cloned from the variable domains of the heavy and light chain of a monoclonal antibody (mab, Fig. 1). The transmembrane domain is taken from CD28 or CD8 or other membrane receptors. CAR T cells contain as signalling domain usually portions of the CD3ζ chain. Activation of CAR NK cells can be acchieved e.g. via the DAP12 chain [6]. Interestingly, the DAP12 chain works equally well in T cells (Bachmann, unpublished). The activation motif in CAR constructs comes either alone or in combination with one or more co-stimulatory motif(s) [e.g. 34, see also Fig. 2]. Costimulatory motifs are commonly taken from CD28 or 4-1BB (CD137) but also from ICOS and OX40 (CD134) [e.g. 2,3,4,5]. CARs containing only the signalling motif of the CD3ζ chain are also known as first generation CARs. CARs containing in addition one costimulatory domain are termed as second generation CARs. CARs with two additional co-stimulatory motif(s) are known as third generation CARs (Fig. 2). In the meantime even more sophisticated CARs have been described which are able to modulate the tumor environment by co-transduction with a gene encoding for an inflammatory cytokine [7]. Alternatively, the signalling and costimulatory domains can be separated to two CARs allowing combinatorial also known as gated targeting of tumor cells [e.g. 8].

It is commonly known that native CD8 and CD28 receptors exist as dimers. Consequently, CARs containing the transmembrane domain of either CD8 or CD28 do also form dimers on the cell surface (Fig. 3) [3]. As schematically shown in Fig. 3, domain swapping of neighbouring scFvs on the surface of CAR T cells can occur. The extent of domain swapping is an intrinsic feature of the respective scFv domain and thus varies between different scFvs. However, the length of the extracellular linker can also influence it [9]. E.g. A shorter spacer between the transmembrane and scFv domain of a CAR can facilitate the interaction

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https://doi.org/10.1016/j.imlet.2019.05.003
Received 23 January 2019; Received in revised form 3 May 2019; Accepted 6 May 2019
Available online 12 May 2019

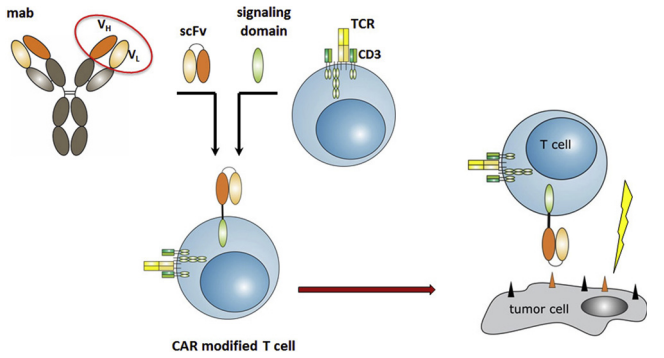

Fig. 1. CAR concept. A conventional CAR consists of extracellular, transmembrane and intracellular signalling domains. The extracellular domain recognizes the TAA on the surface of the tumor cell. It is commonly cloned as single chain fragment variable (scFv) from the variable domains of the heavy (V_H) and light chains (V_L) of a mab. The CAR gene can be inserted into the T cell genome e.g. by viral transduction. The resulting CAR T cell can recognize tumor cells via its ab domain. After cross-linkage the CAR T cell will be activated via its signalling domain(s) and finally destroy the target cell. Both CD4 and CD8 T cells work as killer cells.

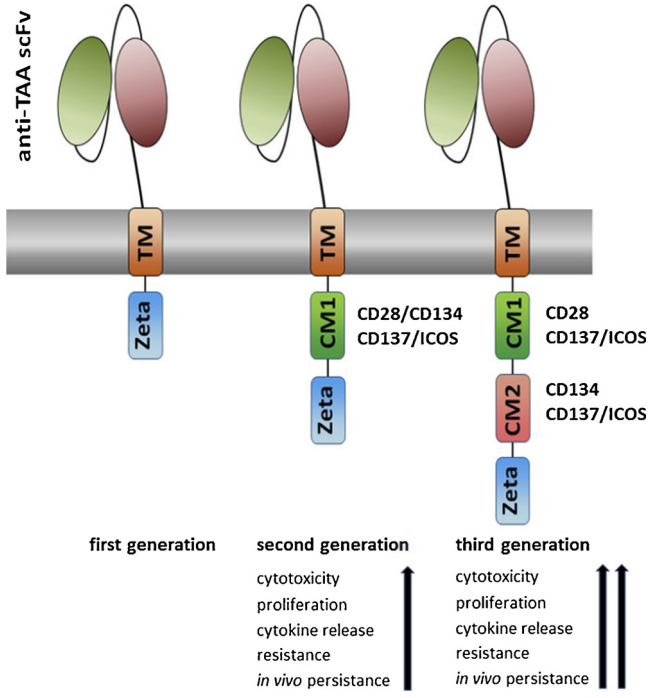

Fig. 2. Different CAR structures. First generation CARs uses as signalling domain the zeta chain of the CD3 complex. To improve their survival and reactivity second and third generation CARs were developed which contain besides the activation additional costimulatory motifs. (CM, costimulatory motif; TM, transmembrane domain).

between the heavy and light chains of neighbouring scFvs. The resulting domain swapping can tremendously effect the tonic signaling in CAR T cells leading to a more or less pre-activated status of the CAR T cells [9]. E.g. A CAR with a strong tonic signal if expressed in a hematopoietic stem cell can result in a complete loss of developing CAR B and CAR T cells [9].

Now after almost 30 years of basic research the clinical application of CAR T cells have underlined their tremendous therapeutical capabilities [e.g. 10–22]. The impressive clinical responses in patients with hematological malignancies resulted recently in the approval of two anti-CD19 CARs by the US Food and Drug Administration (FDA) for the treatment of relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL).

In spite of their success, current CAR T cell therapies are still far away from a routine, safe treatment [e.g. 23]. Patients can suffer from serious or even life threatening side effects such as cytokine release syndrome (CRS) and tumor lysis syndrome (TLS) which prevent a broader application of the CAR technology especially for solid tumors. For instance, the expression of most if not all tumor associated antigens (TAAs) is not limited to tumor cells leading to the risk of on-target/off-tumor effects. This is even true for CD19 as it is not only expressed on leukemic cells but also on healthy B cells. Consequently, CD19 CAR T cells lead not only to the elimination of leukemic cells but also of healthy B cells. In this respect CD19 is just an ideal TAA for CAR T cells as the loss of B cells is not life threatening but can be overcome by application of antibodies (abs). However, such a rescue of healthy tissue function is rather the exception of the rule as most of the TAAs are also accessible on the surface of non-malignant cells of vital tissues.

In order to increase the safety of the CAR technology and to limit such on-target/off-tumor toxicities but also other potentially life threatening side effects, a series of strategies have already been described including for example the use of suicide genes, inhibitory CARs or the targeting of co-expressed surface antigens or an inducible apoptosis switch based on the CRISPR/Casp9 system [24–28]. All of these safe guards have in common that they are designed to eliminate the CAR T cells in case they behave improperly. However, there are obvious limitations of all of these kind of approaches. In principle, there are two major critical time points during a therapy with CAR T cells when it becomes necessary to adjust or even to stop their activity: (i) Soon, after the adoptive transfer of CAR T cells in case severe, untreatable side effects occur, and (ii) Late, when CAR T cells have eliminated all the tumor cells and then continue to attack cross reactive healthy vital tissue. For obvious reasons, at both time points it is pretty critical and difficult to determine the right moment when to destroy CAR T cells as CAR T cells are individual living drugs responding with respect to proliferation and cytokine production in dependence on the tumor load. Therefore, at the time of adoptive transfer of CAR T cells it is almost impossible to predict how the CAR T cells will respond. An obvious alternative would be to inhibit the activity of the CAR T cells. As the signaling of CAR T cells is mediated via phosphorylation of their ITAM motifs, one obvious idea could be to inhibit their signalling by the application of a tyrosin kinase inhibitor (TKI). However, a TKI or other small molecule with a pharmacological function cannot be applied for the rest of the patient's life. Moreover, the steering of CARs via TKI will depend on their pharmakokinetik, mainly their elimination and biotransformation thus enhancing the complexity of an anyhow complex treatment strategy. For example, tumor patients that were pretreated with Midostaurin - a TKI with long lasting therapeutic effects and being able to inhibit the signaling pathway of the T cell receptor - should not immediately be treated with CAR T cells or bsAbs [29].

Another problem might occur if CAR T cells shall be eliminated via antibody mediated cellular cytotoxicity (ADCC). As shown in Fig. 4, a full size antibody requires up to 48 hs to be enriched in a solid tumor as analyzed by PET imaging. 90 min after the application the majority of the mab is still in the peripheral blood and only little is found in the tumor. Consequently, only CAR T cells present in the peripheral blood are easily accessible and can be rapidly eliminated with full size abs. However, full size abs will not rapidly reach the more critical CAR T cells, meaning the activated, proliferating and cytokine producing CAR T cells located in and attacking a solid tumor and/or healthy tissue. Furthermore, another question might be whether or not a sufficient number of functional NK cells will be available at the immunosuppressive tumor cell site and let them do their job as fast as necessary. Similar problems may occur when a genetic approach should be applied to destroy grazy going CAR T cells. At least in case of CRS and TLS there are reasonable doubts that an excision of the CAR gene might work sufficiently fast enough to stop the detrimental effects of CAR T cells. For all these reasons it appears critical to rapidly eliminate

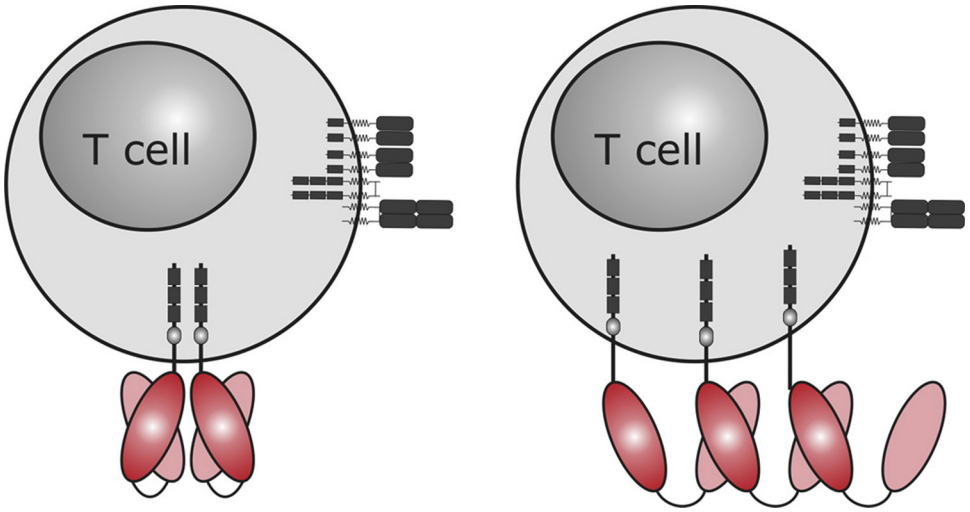

Fig. 3. Dimer formation of CARs. Commonly CAR constructs including UniCARs use the transmembrane domain of CD28. CD28 CARs exist as dimers [3]. Already during the development of first recombinant antibody domains it became evident that two scFvs can form a non-covalently linked dimer by interaction of the heavy chain portion of one molecule with the light chain portion in a second molecule and vice versa [e.g. 61]. A short linker between the heavy and light chain is favourable for such a dimer formation. In CAR constructs we observed that also the spacer length between the transmembrane domain and the scFv can enhance such a domain swapping [9]. The interaction between neighbouring scFvs strongly enhances the tonic signaling in CAR T cells.

CAR T cells e.g. for a treatment of severe CRS or other lethal side effects. It might be even more difficult if CARs targeting more than one TAA are applied to a patient.

So how about an elimination of the CAR T cells after the tumor has been successfully treated? At a first view, an elimination of “retired “CAR T cells appears to be a logical way to prevent them from attacking healthy tissues after all tumor cells have been destroyed. Indeed CD19 CAR T cells could efficiently be eliminated in experimental mice suggesting that such an elimination is feasible at least in principle [28]. However, once again there will be the problem to find the right time point to do so. Even with sensitive imaging tools it will be difficult to determine when to eliminate CAR T cells: If CAR T cells will be destroyed too early some tumor cells may be left leading to recurrent disease. If done too late, on- target/off-tumor effects will occur. Unfortunately, current imaging technologies are not sufficiently sensitive to detect few remaining tumor cells. Even in case improved imaging tools become available, one would still have to wait until it is clear whether or not the CAR T cells can kill these remaining tumor cells. It may well be that the left tumor cells are resistent against the CAR T cells. Consequently, one would have to wait with the decision to eliminate the CAR T cells until there is sufficient evidence that the CAR T cells can no more kill the remaining tumor cells. Again during this time, the patient would suffer just from on target/off tumor side effects. For all of these reasons it would be better to have a more rapid and reliable switch allowing CAR T cells to (i) turn on and off on demand, (ii) change their specificity in case tumor escape variants occur, and (iii) target more than one TAA either simultaneously or subsequently to reduce the risk to select escape variants.

So, how to design such CAR T cells? Looking at natural receptors their function is controlled by interactions with ligands. Consequently, one way to control the activity of artificial receptors could also be the imitation of ligand/receptor interactions. Already in 2012, Urbanska et al. described a modular artificial receptor approach which was based on chicken avidin as artificial extracellular receptor domain instead of an anti-TAA antibody domain of a CAR [30]. T cells modified with such artificial avidin receptors are inactive but can interact with target cells via biotinylated adaptor molecules e.g. biotinylated abs. However, the antigenicity of chicken avidin or bacterial streptavidin and the presence

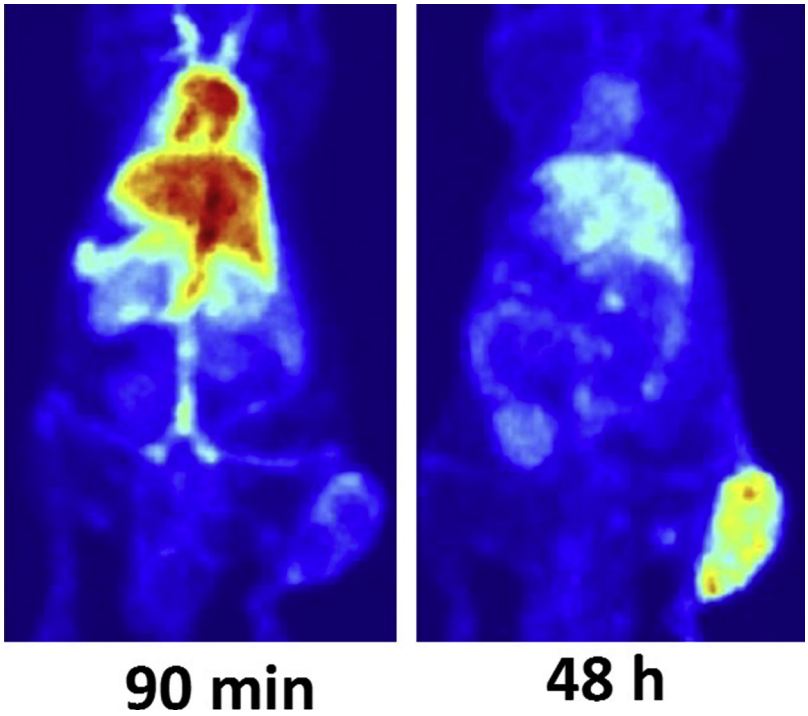

Fig. 4. PET imaging of radiolabelled mabs. Full size abs can well enrich at the tumor site. However, for enrichment in the tumor abs require about 48 h. Even 90 min after injection the majority of the ab is still in the peripheral blood while only traces of it are found in the tumor. Consequently full size abs are not well suitable for a rapid regulation/elimination of adaptor CAR T cells resident in a solid tumor.

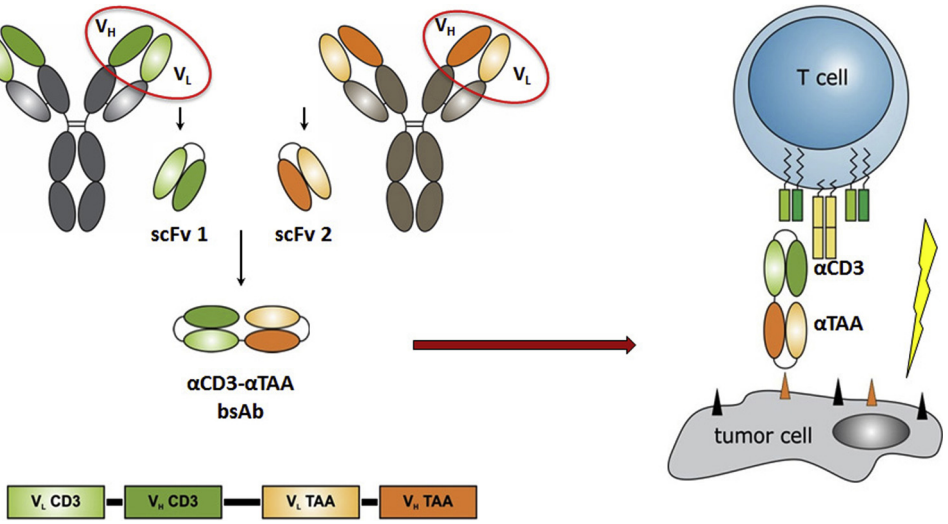

Fig. 5. The BiTE format. BsAbs in the BiTE format consist of two scFvs arranged in a row. One scFv is directed against the CD3 complex, the other scFv is directed against the TAA. Such bsAbs can cross-link T cells and tumor cells which leads to the formation of synapse like structure and finally the elimination of the target cell.

of natural anti-biotin antibodies in sera of healthy individuals [31], might limit the use of such receptors in humans. Moreover, receptors armed with full size abs may behave like conventional CARs due to the long half life of abs. As shown above (Fig. 4) it takes hours for a full size ab to enrich in a solid tumor. Prior to the enrichment into the tumor the abs are present in the circulation where they can last for weeks. Thus, a rapid switch off might not work at least not with adaptor molecules based on full size abs. Moreover, if the target is also expressed on the cell surface of blood or endothelial cells CAR T cells may first attack these cells rather than the tumor cells.

So how to steer more easily the function and side effects of CAR T cells? In parallel to the development of CAR T cells many groups including ours tried to establish bispecific antibodies (bsAbs). The prototype of a bispecific T cell engager (BiTE) Blinatumab (Blincyte®) was recently approved by the FDA [32]. As schematically summarized in Fig. 5, BiTEs consist of two single chain fragment variables (scFvs) obtained from two mabs. One scFv is directed to the CD3 complex of T cells the other one to a TAA on the surface of a target cell. Thus, BiTEs are able to cross-link effector T cells and target cells. Like in case of CAR T cells the cross-linkage via BiTEs leads to the formation of a synapse like structure and finally to the destruction of the target cells [32,33]. During the development of bsAbs [e.g. 34,35,36] we faced the problem that a novel bsAb cannot simply constructed by replacing the anti-TAA domain. Unpredictably, the replacement of the one of the two scFv domains also effected the properties of the unchanged second scFv domain [34]. In order to compare the efficacy of different ab domains originally we designed a modular bsAb format termed UniMAB [37–40, Fig. 6]. For that purpose we split the original BiTE into two components: (i) an effector molecule (EM) and (ii) a target molecule (TM). The EM itself is a universal bsAb. On the one hand it is directed to CD3 on the other hand to a peptide epitope. First TMs were fusion molecules consisting of an anti-TAA scFv and the peptide epitope [e.g. 38,39]. Consequently, EM and TM can form a bispecific immune complex. Like BiTEs it can bind to CD3 and a TAA. Most of interest, such immune complexes can functionally replace conventional bsAbs [38–40]. Aside of monovalent TMs proof of concept could also be shown for TMs that were constructed as bispecific bivalent molecules or were fusion molecules containing a costimulatory domain [Fig. 7, 39,40].

The next logical step was to create a CAR based on the same anti-epitope scFv originally used in the EM of the UniMAB system. So the same TMs shown to work in the modular BiTE system could immediately be combined with this anti-epitope CAR. The resulting modular CAR system was termed UniCARs (Fig. 8). It was first presented at the ASH meeting in 2014 [41]. Since then other related switchable CAR strategies (e.g. sCARs) were published [42–44].

The target epitope of both the modular EM and the UniCAR system is physiologically not accessible on the surface of any living cell neither tumor nor healthy cell. Consequently, after adoptive transfer of such UniCAR T cells into a patient they will remain silent (Fig. 8, OFF). However, a cross-linkeage with tumor cells via a TM leads to the activation of the UniCAR T cell (Fig. 8, ON). Most importantly, UniCAR T cells can be repeatedly turned “on “simply by infusion of the TM and turned “off “by stopping of the infusion and elimination of the TM. Obviously, the prerequisite for a safe and fast steering of the UniCAR system is that the TM will be rapidly eliminated in case severe site effects occur. As shown by PET analysis TMs based on scFvs or nanobodies for example full fill this prerequisite [e.g. 45–50]. Such TMs have usually elimination half lives of about 15–45 min. The downsite of this strategy is that the respective TM has to be given by continous infusion. Thinking of treatment times of several weeks this appears inconvenient for patients. However, one could start a UniCAR therapy with a TM having a short half life and continous infusion. Once most of the tumor burden is destroyed and thus the risk of CRS and TLS will be low one could switch to a TM having an extended half life. For example IgG4 based TMs have a kinetic comparable to full size abs and show such an extended half life (Fig. 9). Until now, we have already developed a series of TMs with short and extended half life. For both kind of TMs we have shown proof of concept *in vitro* and in experimental mice for retargeting of UniCARs to a variety of targets including for example to CD19, CD123, CD33, PSCA, PSMA, GD2, EGFR, MUC1, STn, BCMA, and others [41,45–51], Bachmann unpublished)]. According to these studies TMs can be constructed from scFvs derived from the heavy and light chain of murine or humanized mabs but also from camelid abs so called nanobodies (Fig. 10). As already described for the modular BiTE format, multivalent or multispecific TMs can be created e.g. by fusion of two scFvs or nanobodies or combinations thereof via the UniCAR peptide linker. Simultaneous or subsequent application of different monospecific TMs or application of bispecific TMs can easily be used for (OR) gated targeting to reduce the risk of selection of tumor escape variants [45,51]. Interestingly, TMs are not limited to ab derivates. Aside of affibodies and soluble TCRs, even small molecules can be converted to TMs (Fig. 10, Bachmann unpublished). For example, fusion of the UniCAR epitope to commonly used PET tracers allowed us to establish a novel class of theranostic compounds that can be used for both immunotherapy (retargeting of UniCAR T cells) and simultaneously for PET imaging to follow the CAR T therapy in an

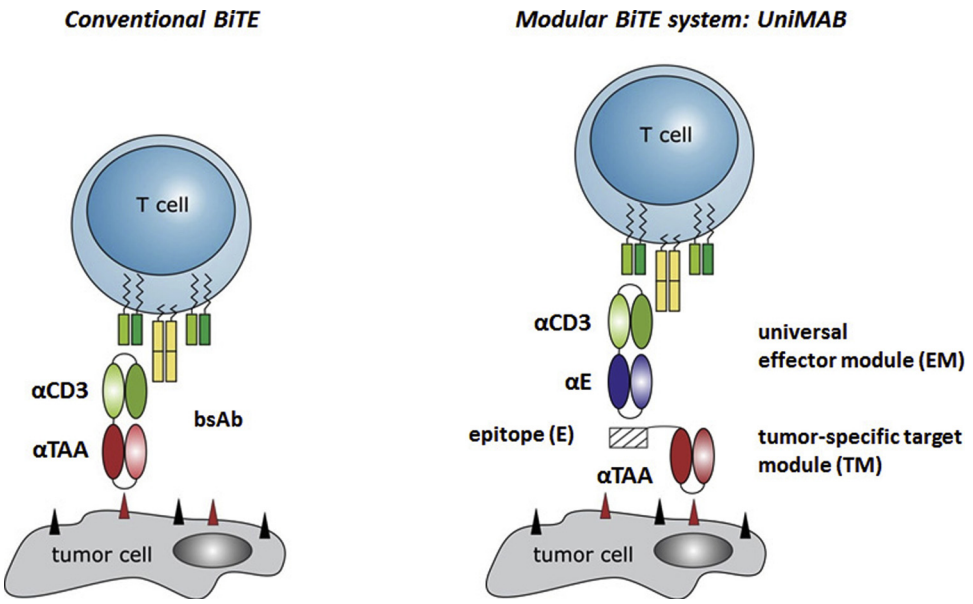

Fig. 6. UniMAB, a modular BiTE format. During construction of novel BiTEs we faced the problem that minor changes in one ab domain also affected the untouched second ab domain. In order to compare different scFv domains more reliably we developed a modular BiTE format (UniMAB). We split the bsAb in two components. An effector module (EM) and a target module (TM). The EM is a universal bispecific ab that can be combined with any TM. For that purpose it is directed on the one hand to the CD3 complex and on the other hand to a peptide epitope. A TM is a bifunctional molecule which on the one hand recognizes a TAA on the surface of a tumor cell and on the other hand contains the peptide epitope. EM and TM can form an immune complex which behaves similar to a conventional BiTE and can cross-link T cells with tumor cells [e.g. 38–40].

individualized manner (Bachmann,unpublished).

So the key to the UniCAR technology is the selected peptide epitope. The UniCAR epitope is taken from the human nuclear autoantigen La/SS-B [52]. As shown in Fig. 11, the epitope is part of a random coiled linker between the N-terminal La motif and the first RNP consensus sequence of the La/SS-B protein. The linker is cryptic in native La protein as the anti-La mab against this epitope cannot precipitate native La protein. Only after heat denaturing during SDS-PAGE/immunoblotting the epitope becomes accessible. In contrast, synthetic peptides or epitope fusion proteins react with the anti-La mab. So why did we select an epitope from an autoantigen for such a purpose? Isn't there the risk that it can trigger an autoimmune response? Over the past decades we and many other groups have analyzed the structure, function, expression, and also the immune response against the nuclear autoantigen La/SS-B including in anti-La positive autoimmune patients [e.g. 53,54,55,56]. In none of these studies an immune response against the UniCAR epitope either at the B- or T cell level was seen. Consequently, even autoimmune patients being able to break tolerance against the La antigen do not develop anti-La abs against the UniCAR epitope. Thus it appears rather unlikely that an individual being

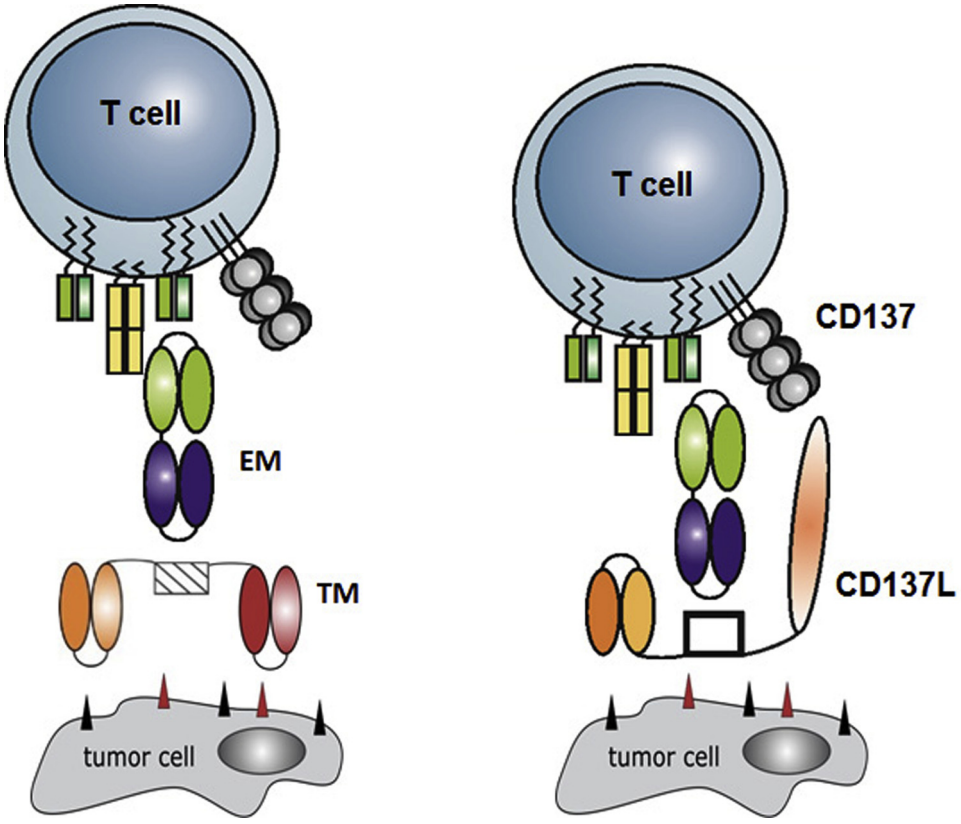

Fig. 7. Multifunctional target modules. First TMs were based on a monovalent scFv. However, bispecific bivalent, monospecific bivalent TMs and combinations of a scFv with a costimulatory domain are also functional [39,45,51].

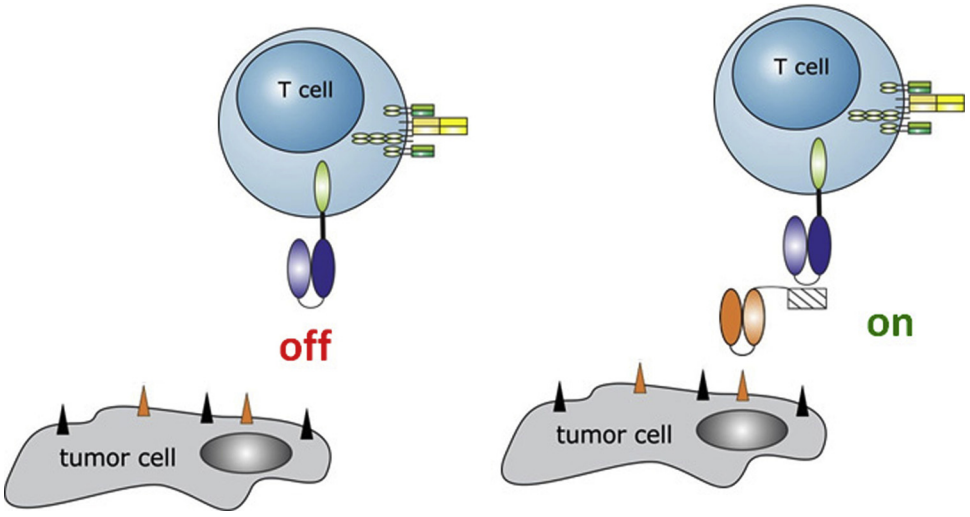

Fig. 8. UniCAR, a modular switchable CAR platform. Based on the anti-peptide epitope scFv used in the EM of the modular BiTE format UniMAB (see also Fig. 7) we next constructed a universal CAR domain termed UniCAR. UniCAR T cells are inert as the recognized peptide epitope is physiologically not present on the surface of cells either on healthy or tumor cells [e.g. 37]. However, they can be redirected to tumor cells in the presence of a TM equipped with the peptide epitope (UniCAR epitope). Consequently, UniCAR T cells can be turned ON in the presence of a TM and will automatically shut OFF once the TM is eliminated. For a fast switch the TM must rapidly enrich at the tumor site and also rapidly be eliminated in case of an emergency shut down. TMs based on scFvs, nanobodies, bivalent formats thereof and even small molecules fulfill this prerequisite [41,45–51].

tolerant to the La antigen will develop an immune response against the selected La epitope. But even in the worst scenario that we would trigger an anti-La autoimmune response by the UniCAR epitope, the UniCAR epitope is cryptic in native La protein. Moreover, if the anti-La response spreads to other La epitopes, anti-La abs are thought to be protective against anti-DNA abs in lupus patients [57]. Another advantage of the selected UniCAR epitope is the primary sequence of La protein is conserved during evolution including in rodents [58], so the toxicology of UniCARs can easily be addressed in mice.

In summary, safety issues which currently limit a common application of the CAR technology especially in solid tumors may be overcome by modular CARs such as the UniCAR system.

2. Future direction

No doubt the development of checkpoint inhibitors, bsAbs, and the CAR technology has shown the tremendous potential of immunotherapeutic approaches. However, their clinical application also told us about their tremendous risk to cause even life threatening side effects. Conventional CAR T cells cannot be turned off once adoptively transferred into patients. To overcome this limitation until today a variety of strategies have already been put forward including modular CAR strategies. Certainly, only the clinical application of modular CAR strategies such as the UniCAR system which will hopefully start in the mid of this year will tell us if these kind of CAR strategies are really superior. Fairly to mention that steering the activity of CAR T cells is only one of the problems that still need to be solved. A series of additional questions remain. For example, how to efficiently attract the immune cells into a solid tumor and how to circumvent the immunosuppressive tumor microenvironment? A further question is, will there be an advantage to use certain subpopulations of T cells? Although both CD8 and CD4 T cells can kill target cells when engaged via either CARs or bsAbs, one should keep in mind that genetically modified PBMC preparations will also contain CAR Treg cells. The same is true for the engagement of T cells via BiTEs. Would it be superior to remove them? At least from the clinical response of CAR T cell and also from BiTE therapies it is obvious that Tregs co-activated in the context of these therapies cannot completely inhibit the effector T cells. So how

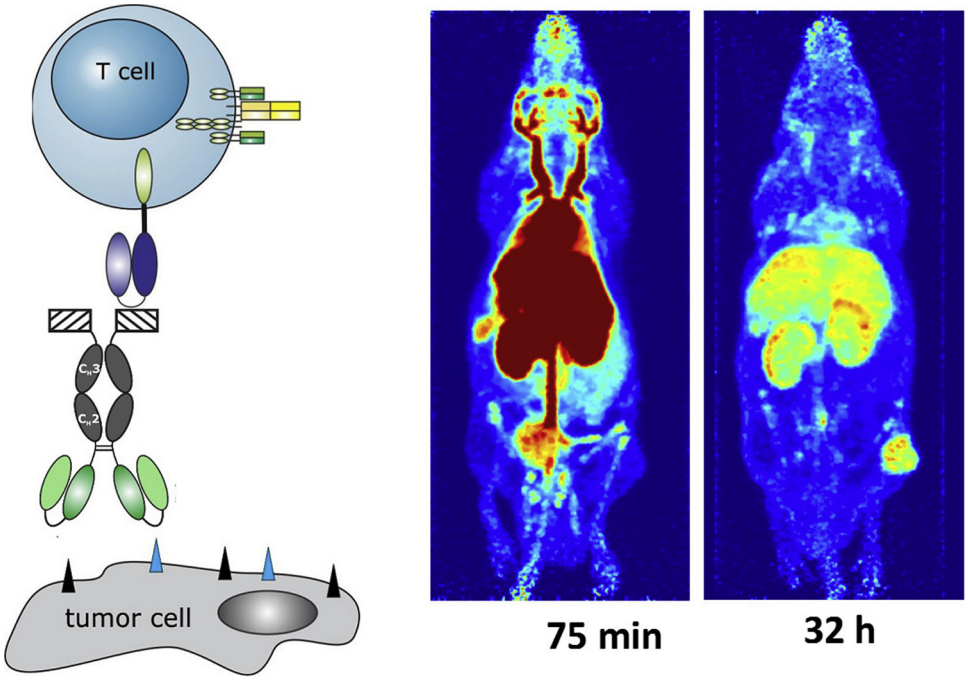

Fig. 9. Target modules with extended half life. A rapid regulation of UniCAR T cells requires short half lifes of TMs. Due to their long half life (see also Fig. 4) UniCAR T cells armed with TMs based on full size mabs for example will behave more like conventional CARs. Such TMs are not suitable for an emergency shut down if necessary. However, once most of the tumor load has been destroyed and the risk of CRS and TLS will be low then TMs with extended half life would be more convenient for the patients as their application would not depend on permanent infusion. Therefore, we also constructed TMs with extended half life. E.g. IgG4 based TMs fulfill this requirement as analyzed by PET.

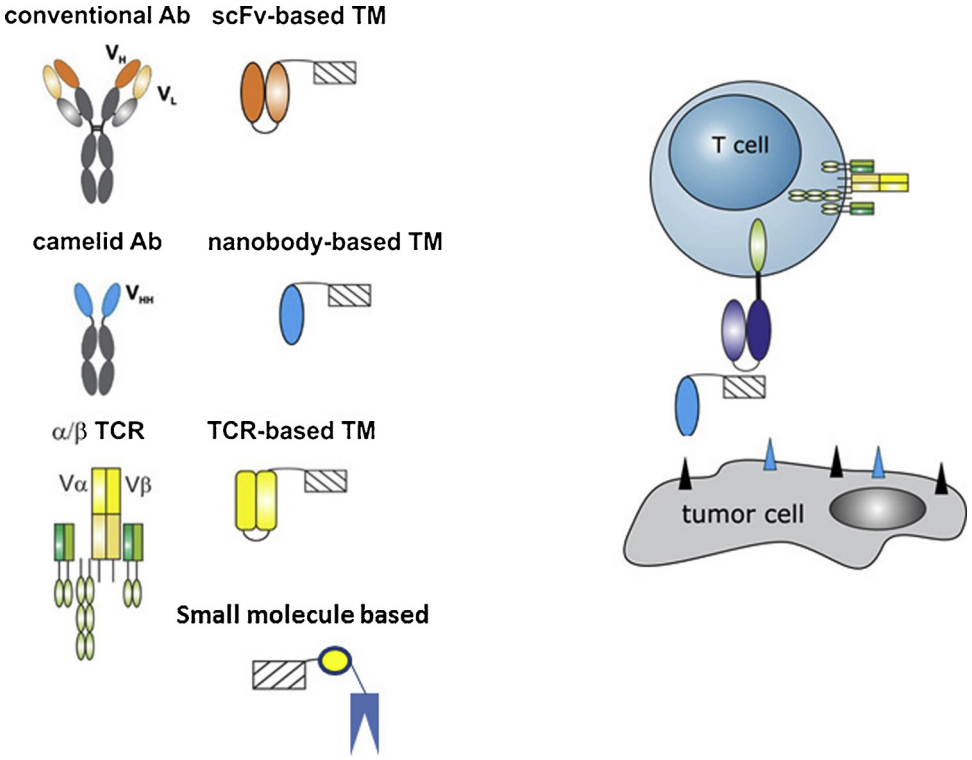

Fig. 10. Target modules for UniCAR T cells. Originally TMs were based on scFvs derived from mabs. In the meantime we also created TMs from a variety of molecules including nanobodies, affibodies, soluble TCRs, and even small molecules [41,45–51], and Bachmann unpublished)].

to further improve T cell based immunotherapies? For example external beam irradiation or endoradionuklide therapy may help to attract immune cells into the tumor tissue and the disintegrated cells may lead to additional vaccination effects. In addition, combination of checkpoint inhibitors with CAR T cells or BiTEs may further improve these therapies by releasing the brakes of the immune system. Also, the combination with small molecules may be of interest. For example, special TKIs can lead to an upregulation of potential cell surface targets (e.g. FLT3) and may therefore be supportive as long as they do not destructively inhibit the function of (CAR) T cells [29]. Like all monotherapies one has to expect that resistant tumor cells will be selected when CAR T cells or BiTEs with a single specificity will be

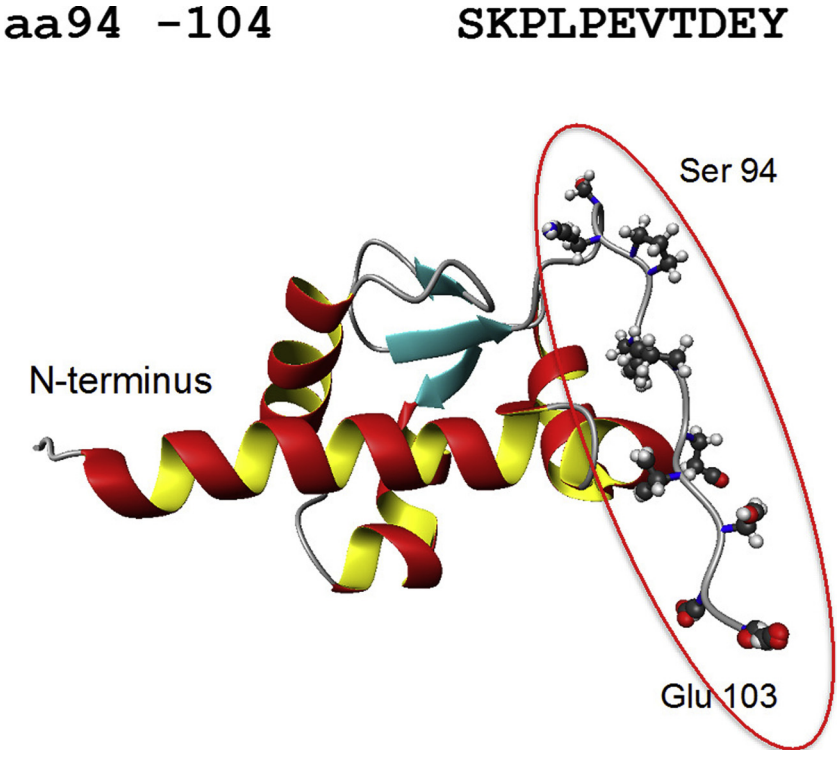

Fig. 11. The UniCAR epitope. As UniCAR epitope we selected the amino acid sequence (aa) SKPLPEVTDEY (or the shortened version KPLPEVTDEY as the serine is not necessarily required) which represents the aa94 -104 of the human nuclear autoantigen La SS-B. This aa sequence is a random coiled portion of the La motif which links the La motif with the first RNP1 consensus sequence in the La protein.

applied. Conventional CAR T cells with dual specificities have already been introduced into the clinics but the risk of side effects may even be higher. Again modular strategies may be superior allowing to target more than one tumor target simultaneously. In case of the UniCAR system for example more than one TM can be applied sequentially or simultaneously. That way "OR"-gated targeting can easily be realized by monospecific TMs but also by using bi- or even multispecific TMs. Especially in case of "AND" gated targeting strategies a lot of fine tuning work might still become necessary as it depends not only on the affinity of the CAR components but also on the number of binding sites at both the effector and the target cells. Another advantage of modular approaches is that TMs can be created containing costimulatory domains [40]. Of cause modular CAR strategies such as the UniCAR approach have not only advantages: The prerequisite for a rapid shut down is a short half life of the TM. The consequence is that the TM has to be given continuously by infusion. One alternative could be the application of TMs with extended half lifes. However, such TMs would not allow a rapid shut off. Consequently the most convenient solution for a patient would be to have two TMs with the same specificity: (i) One with a short half life for optimal rapid steering if necessary at the beginning of the treatment, and (ii) another one with an extended half life that could be given once most of the tumor cells are eliminated. The obvious draw back of this strategy will be the cost of the two TMs. Another possibility would be to produce the TM in situ. For example we managed to encage genetically modified cells in cryogels. After implantation they were able to produce in situ recombinant antibody derivates including a UniCAR TM [59,60]. To further improve the efficacy especially in solid tumors, UniCAR T cell cells could be modified to secret cytokines or provide costimulatory molecules as elegantly shown for conventional CARs [7]. Finally, in order to further improve the safety and efficacy of such immunotherapeutic approaches theranostic imaging tools are needed that are based on the same paratopes as used for immunotherapy.

Conflict of interest

M. B. has invented the modular BiTE and the UniCAR system and holds filed patents related to BiTEs and the UniCAR system. In addition he is founder and shareholder of the company GEMoaB which ows the IP related to the UniCAR system.

Acknowledgments

I like to thank to all of my former and current Bachelor, Master, Diploma, PhD and MD students, Postdocs, and technicians for their enthustiastic support. Especially Prof. Dr. M. Schmitz, one of my first doctoral students, opening us the field of T cell immunology; Dr. H. Bartsch, who characterized the La gene(s) expression; Dr. A. Feldmann, who established the first BiTE in my lab; Dr. A. Feldmann, Dr. N. Berndt and Dr. C. Arndt, who established and compared the modular BiTE system with conventional BiTEs thereby also setting up the prerequisites for the UniCAR system; Prof. Dr. H. Abken, Prof. Dr. A. Temme and Dr. M. Cartellieri, who helped us to establish the conventional CAR T cell technology in our lab; Dr. S. Koristka, who presented the UniCAR system for the first time at the ASH meeting in 2014; Prof. Dr. R. Bergmann for providing PET imaging data; Prof. Dr. E. P. Rieber, who convinced me to return from the U.S.A. back to Germany and stimulated the development of our first BiTEs; all coworkers of the spin out companies GEMoaB and Cellex PT for the development of clinical grade GMP products based on our BiTEs and the UniCAR system; all my former colleagues and friends at the OMRF, Drs. B. Kurien, K. Kaufman, Profs. Drs. H. Scofield, A. D. Farris, J. A. James, J. B. Harley, and especially my personal friends and technicians Tim and Jody A. Gross who helped me to characterize our anti-La mabs. In addition, I like to thank Prof. Dr. G. Ehninger for his friendship and his enthustiastic support and effort to drive our BiTEs and the UniCAR system forward into the clinic. I also like to thank the NIH, OCAST, DFG, BMBF, Jose Carreras-, Sander-, Miltrid Scheel-, Dr. Robert Pfleger-, VW-, and Thyssen Stiftung for their financial support.

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