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Review

TCR-MHC/Peptide Interaction: Prospects for New Anti-tumoral Agents

ULRICH H. WEIDLE, GUY GEORGES and GEORG TIEFENTHALER

Roche Pharma Research and Early Development (pRED), Roche Innovation Center Penzberg, Roche Diagnostics GmbH, Penzberg, Germany

Abstract. *Tumor-related antigens can be presented as peptides forming complexes with major histocompatibility complex (MHC) molecules that interact with T-cell receptors, thus generating an immunologic anti-tumor response. Unfortunately, however, this response can be decreased by many effectors and pathways. On the other hand, such peptide-MHC complexes are unique starting points for therapeutic intervention. We present strategies for eliciting an anti-tumoral response by T-cell receptor-based fusion proteins with interleukin (IL)2 and antibody constant region domains, superantigens, and T-cell recruiting antibodies, as well as using genetically modified autologous T-cells as effectors. Another strategy is to direct peptide-MHC complexes to tumors as fusion proteins with an antibody-derived targeting moiety. Finally, we describe T-cell receptor-mimicking antibodies and antibody conjugates as anti tumoral agents.*

Tumor-related antigens can be expressed by tumor cells either directly as plasma membrane-associated proteins (transmembrane or glycosylphosphatidyl-inositol-anchored) or as peptides complexed with major histocompatibility complex (MHC) class I molecules (1). The latter consist of three non-covalently associated components: an MHC-I α-chain, a β2-microglobulin light chain and a peptide ligand comprising of 8-12 amino acids (2). Proteins from all cellular compartments can be degraded and processed into peptides by the proteasome and subsequently be transported into the endoplasmic reticulum (ER) where peptide MHC I (pMHC I) complexes are assembled to be finally integrated into the cell membrane for interaction with T-cell receptors (TCR) (3, 4). This process enables the immune system to eliminate tumor cells based on the cell surface presentation of cancer-related peptide antigens. Unfortunately, formation and surface expression of these complexes can be interfered with at many levels, such as down-regulation of MHC I molecule expression (5). Therefore, conservation and/or reconstitution of functional MHC-peptide complexes on the cell surface are paramount for the induction of a T-cell-based anti-tumoral response. In the following, we describe strategies for reconstituting and complementing this process using TCR-based fusion proteins, antibody-pMHC complexes, antibody-based TCR mimetics and genetically engineered T cells.

Correspondence to: Georg Tiefenthaler, Roche Pharma Research and Early Development (pRED), Roche Innovation Center Penzberg, Roche Diagnostics GmbH, Nonnenwald 2, D-82372 Penzberg, Germany. Tel: +49 8856602343, Fax: +49 885660792343, e-mail: georg.tiefenthaler@roche.com

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TCR Fusion Proteins

TCR-IL2 fusion protein ALT-801. The rationale for the design of ALT-801, a single-chain (sc) TCR-IL2 fusion protein (Figure 1A), was based on two observations. First, interleukin 2 (IL2) is a cytokine involved in the expansion of T-cells, natural killer (NK) cells and lymphokine-activated killer cells (LAK), thereby promoting anti-tumoral activity. Unfortunately, however, standard dose IL2 treatment induces acute toxicity partly due to vascular leak syndrome thus restricting its use to relatively healthy patients (7) and, as a consequence, argues for localized expression of IL2 at the tumor site. The second observation is a strong over-expression of p53 in most tumors compared to normal tissues (8). Specifically, the p53-derived antigenic peptide covering amino acids (aa) 264-272 is found in complex with MHC I molecules in several types of tumors (9). Consequently, ALT-801 was designed as a bi-functional fusion protein comprising IL2 linked to a soluble scTCR that recognizes peptide 264-272 of human p53 on cancer cells in the context of HLA-

A*0201 (10). High *in vivo* efficacy of ALT-801 was demonstrated in p53+/HLA-A2+ human melanoma (A375), breast cancer (MDA-MB231) and pancreatic carcinoma (PANC-1) xenograft models (10-12). Based on these findings, ALT-801 was evaluated in a phase I study in patients with advanced malignancies (13). The serum half-life ranged between 2 and 5 h, depending on the dosing time and, thus, was significantly longer than that of unmodified IL2. Side-effects were similar to those observed after bolus treatment with IL2, but less severe. In addition, ALT-801 treatment induced an increase in serum interferon IFNγ. With respect to efficacy, 10 patients out of 26 showed stable disease for at least 11 weeks, while one complete response of a patient with metastatic melanoma was observed. ALT-801 was found to activate cluster of differentiation 4+ (CD4+) and CD8+ T-cells. In addition, these infiltrating T lymphocytes secrete IFNγ into the tumor microenvironment and, thus, can potentially repolarize tumor-associated macrophages by promoting the M_2 phenotype and eradicating the M_1 phenotype (www.altorbioscience.com). Additional clinical studies have been initiated to investigate the efficacy of ALT-801 in combination with cisplatin in patients with metastatic melanoma, with gemcitabine and cisplatin in patients with metastatic bladder cancer or as a monotherapy in patients with relapsed or refractory multiple myeloma (14) (www.altorbioscience.com). Similar to ALT-801, ALT-802 is a fusion protein consisting of the above-mentioned scTCR specific for p53 and the human immunoglobulin (Ig)G1 heavy chain constant region including the fragment crystallizable (Fc) region, which is able to mediate antibody-dependent cell-mediated cytotoxicity (ADCC) (9). In a model of experimental non-small cell lung carcinoma (NSCLC) metastasis in nude mice, the fusion protein containing the heavy chain constant regions CH1-CH2-CH3 was more active than the CH2-CH3 containing version. Moreover, improved *in vivo* efficacy was observed when the treatment was combined with cisplatin (15). A clinical phase I study for this molecule is planned for treatment of p53+ NSCLC patients. Under preclinical development are p53scTCR molecules fused to an IL15 superagonist mutein complexed with IL15Ra-Fc possessing immunostimulatory activity (16, 17). In addition, non-p53 targeted effector molecules specific for tumor-associated antigens, such as melanoma antigen gene-3 (*MAGE-3*), glycoprotein 100 (gp100) and melanoma antigen recognized by T cell 1 (MART-1) are evaluated preclinically (www.altorbioscience.com).

Superantigen TCR fusion proteins. Superantigens (SAgs) are a group of antigens, which activate T-cells in an antigen-independent manner resulting in oligo- or polyclonal T-cell responses. In contrast to a normal antigen-specific T-cell response, which comprises 0.001% to 0.0001% of T-cells, up to 10-20% of TCRs are engaged by SAgs (18-20). SAgs are mostly expressed by pathogenic bacteria or viruses. In a first step, SAgs bind to MHC II molecules on antigen presenting cells (APC) outside the peptide binding groove and subsequently interact with defined classes of α- or β-variable domains of TCRs, depending on the respective SAg. Affinity of the SAg for the TCR correlates with the strength of T cell activation (21, 22). Secretion of cytokines upon SAg-induced TCR stimulation, with IFNγ as a prominent mediator, subsequently leads to macrophage activation which in turn overproduce pro-inflammatory cytokines such as IL1, IL6 and tumor necrosis factor α (TNFα) (21, 22), which can also eliminate antigen-negative tumor cell variants.

The basic idea for designing fusion proteins composed of an antibody-related moiety targeting the molecule to tumor cells and a SAg is the functional similarity to a genuine TCR-pMHC interaction, which results in an antigen-independent recruitment and activation of T-cells and, consequently, a polyclonal T-cell response. High affinity antibodies to the tumor antigen and low affinity interactions of TCR-pMHC in the micromolar range are characteristic features of these molecules. Thus, decoration of tumor cells with SAgs mimicking a bacterial infection induces an inflammatory response at the tumor site. In a seminal work, a recombinant fusion protein consisting of staphylococcal enterotoxin A (SEA) and a monovalent antigen binding fragment (Fab) of monoclonal antibody (mAb) C215 (Figure 1B), which is specific for human colon carcinoma cells was evaluated (23). SEA, in the context of the fusion protein, had a ≥10-fold reduced MHC II binding as compared to native SEA and the affinity of the Fab C215-SEA fusion protein was 100-fold stronger for the C215 tumor antigen than for MHC II. Functionally, the fusion protein was able to induce T cells to lyse C215+ MHC II- human colon carcinoma cells. In mice transplanted with B16 melanoma cells, which had been transfected with the C215 antigen, treatment with the fusion protein resulted in inhibition of tumor growth and long-term survival (23). The clinically most advanced SAg fusion protein is Naptumomab estafenatox (ANYARA, ABR-217620) (24), which is presently being tested in phase III studies in patients with renal cell carcinoma (RCC) by Active Biotech. This fusion protein is composed of a Fab fragment directed against the 5T4 oncofetal antigen (25, 26) and a SAg based on staphylococcal entertotoxin E (SEE) containing modifications that limit systemic, cytokine-induced toxicity and reduce immunogenicity. Basically, ANYARA was designed by reducing the affinity of ABR-214936 (5T4FabV13-SEAD277A), which was undergoing phase II studies to MHC II, by 100-fold to decrease potential systemic toxicity (24, 27). This resulted in a molecule that could be administered at doses in the microgram per kg/patient range as compared to the nanogram doses of the previous version. In addition, immunogenicity was reduced by creating a chimeric SAg (SEA/E120). Specifically, amino

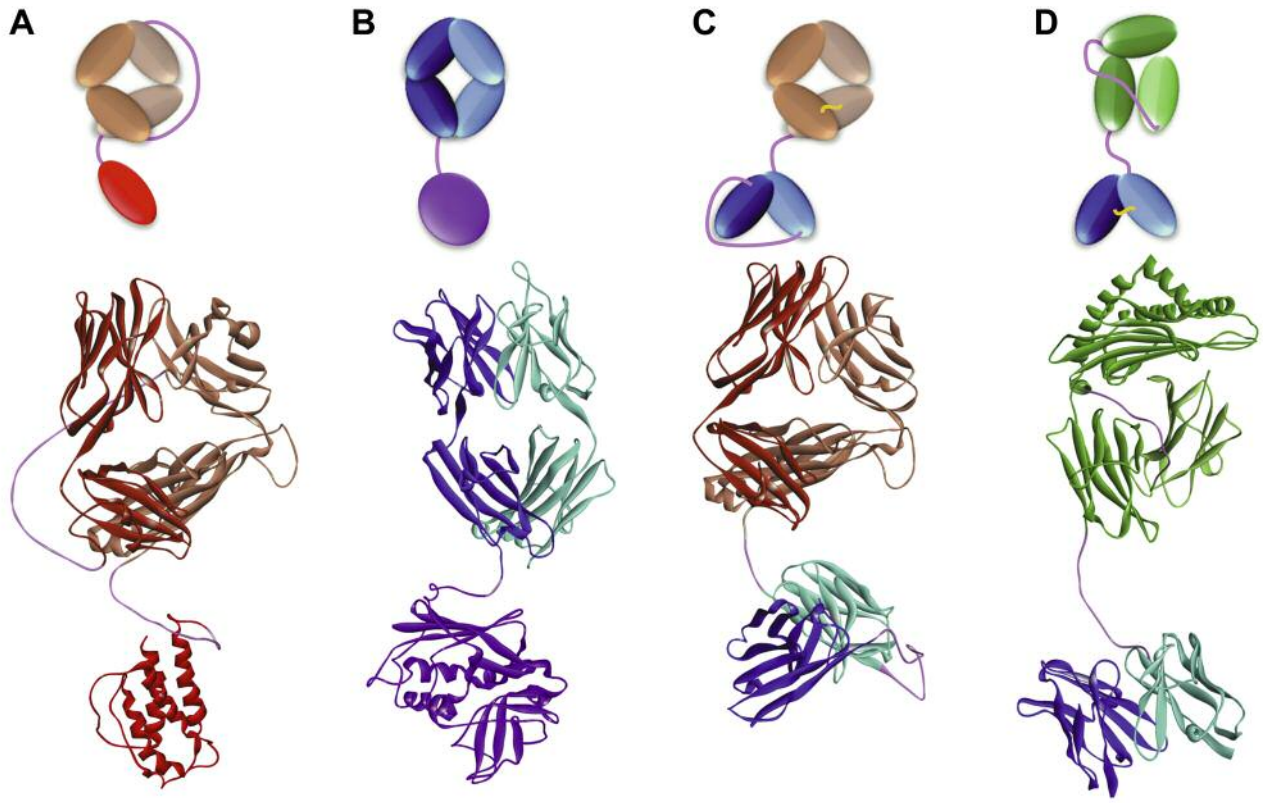

Figure 1. *Schematic and 3D representation of selected TCR-MHC targeting anti-tumor agents. Schematic representations are shown in the upper panel, the corresponding 3D representations are depicted in the lower panel. Structure models were generated based on available structure data of domains or entities. Structural data from the Protein DataBank (PDB, Nov. 2011) (129) were assembled and minimized using DiscoveryStudio40 (129). Ribbon representations display secondary structures where the N-terminal domain is placed on the top and the C-terminal domain on the bottom. A TCR assembly is composed of two chains, α and β, displayed in dark and light brown, respectively. The targeting antibody heavy and light chains are represented by dark and light blue ribbons, respectively. An MHC complex comprises a dark green α chain combined with the light green β2 microglobulin domain. Fusion linkers and additional disulfide bridges are shown on the schematic view in pink and orange, respectively. A. Model of the ALT-801 TCR-IL2 fusion molecule (pdbcode: 4P2R for TCR and 2GFB for IL2, displayed in red). B. Model of ABR-217620 comprising a targeting Fab-moiety and the Staphylococcal enterotoxin A shown in purple (pdbcode: 1VPO and 1LO5, respectively). C. Model of an ImmTAC comprising a soluble TCR (pdbcode: 2P5E) stabilized by a disulfide bridge and fused to an anti-CD3 scFv (pdbcode: 2FGW). D. Model of a scMHC (pdcode: 4MNQ) fused to an anti-IL2R Fv (pdcode 3NFP) stabilized with a disulfide bridge.*

acid residues interacting with MHC II and TCR were engineered into the backbone of SEE. The resulting fusion molecule ABR-217620 interacts with TCR β variable chain family members 7-9 and binds to the 5T4 antigen on tumor cells (28). It both possesses a high affinity for tumor cells (Kd=1nM) and mimics a genuine TCR-pMHC contact in the 1 μM affinity range. ABR-217620 has insufficient affinity to allow a significant monomeric T-cell interaction, thus avoiding binding to, and blocking or triggering of, T cells as a monomeric target structure. A respective phase III study encompassing 513 RCC patients was concluded in January 2013 (www.activebiotech.com). In a hypothesis-generating subgroup analysis, there was a trend towards a survival benefit by ANYARA treatment for those 25% of patients with low/normal IL6 levels and anti-superantigen antibody.

ImmTacs (Immune mobilizing monoclonal TCRs against cancer). One of the difficulties of targeting effector T-cells to tumors is immunological tolerance through deletion of T-cells with strong affinity to self-antigens (29). In addition, tumors often have a reduced density of MHC-peptide complexes on the tumor cell surface due to decreased MHC expression and/or antigen processing (30). A strategy to overcome these problems is to redirect T cells to tumors by using fusion molecules consisting of a T-cell recruiting domain and a soluble TCR-based targeting moiety (ImmTacs) (Figure 1C) (31, 32). The therapeutic potential of anti CD3 scFv fragments to function as such T cell recruiting elements was demonstrated in the context of bispecific T cell engagers

(BiTEs) (33, 34). In addition, the targeting domain is a soluble, pMHC-specific, high-affinity (picomolar) monoclonal TCR-derived protein fragment. Antibody engineering techniques, such as the introduction of a disulfide bond into the soluble TCR (35) and phage-display optimisation of the complementarity determining regions (CDRs) of the TCR, have allowed the stabilization and affinity maturation of the respective TCR from micromolar to the picomolar range (36-38). In contrast to antibody-based cancer therapeutics targeting membrane-associated or secreted antigens only, the ImmTac technology also allows targeting of intracellular tumor antigens. For example, many classical tumor antigens, such as New York- Esophageal Cancer-1 (NY-ESO-1), L Antigen-1 (LAGE-1) and human melanoma antigen A-3 (MAGE-3) (39) or tissue-specific antigens, such as gp100 and melanoma antigen A (MEL-A), are located intracellularly (40). The same holds true for tumor-related anti-apoptotic proteins, such as B cell lymphoma 2 (Bcl-2), p53, survivin and Wilms Tumor-1 (WT-1) (41). In addition, peptides derived from mutated inducible enzymes involved in cancer metabolism can be presented by MHC molecules (42). Importantly, in terms of translation into a clinical setting, patients have to be stratified with regard to their human leukocyte antigen (HLA) status.

In practice, bi-specific TCR/anti-CD3 fusion molecules directed against NY-ESO-1, LAGE-1, gp100, MAGE-3 and MEL-A were generated and functionally evaluated (31, 32, 43). It was shown that these agents bound to human tumor cells and were able to activate $CD8^+$ T-cells and that the specific T-cell activation potency correlated with the pMHC I affinity of the respective molecules. In addition, tumor cell lysis induced by such redirected T-cells was shown. *In vivo*, the ability of ImmTacs to inhibit tumor growth was demonstrated in tumor xenograft models using non-obese diabetic severe combined immune-deficient NOD-SCID, SCID-beige or NOD SCIDγ (NSG) mice engrafted subcutaneously with Mel 526, A375, SK-Mel-37 or J82 tumor cells. Here, unstimulated human peripheral blood mononuclear cells (PBMCs) were used as effector cells (31, 32, 34). Remarkably, ImmTacs were able to induce tumor cell lysis at pMHC densities as low as 2-10 copies per cell. Clearly, the potential to target tumor cells with low expression levels of tumor antigens extends the therapeutic potential of engineered protein-based medicines. Finally, the anticipated dose for applications in humans will be probably below 10 mg (31). From a regulatory point of view it should be kept in mind that both tumor and T-cell targeting moieties are specific for the respective human gene products and, therefore, a species for target-related toxicity testing will not be available. On the other hand, immunogenicity of such fusion proteins is probably relatively low since the TCR moiety is fully human, the anti-CD3 scFv is derived from a humanized antibody, the engineered disulfide bond is buried within the protein and the mutations for affinity maturation are introduced into the CDRs.

Ab-MHC complexes. The efficacy of T-cells in clearing viral infections is well known (44). On the other hand, expression of tumor-specific MHC-peptide complexes on the surface of cancer cells can be affected by either down-regulation of MHC I expression or by alterations in the peptide-MHC processing and presentation pathway (30, 45-48). Several groups have shown previously that biotinylated MHC-peptide complexes multimerized by streptavidin or monomeric influenza matrix peptide complexes coupled chemically to tumor antigen-related antibodies were able to induce lysis of tumor cells coated with the respective complexes (49, 50-53). These approaches made use of antibodies or antibody Fab fragments and non site-specific chemical conjugation resulting in limited homogeneity of the produced conjugates and, consequently, variable tumor penetration due to their large size. These problems were addressed by fusing an antigen-specific moiety such as an antibody-derived scFv fragment directed against a tumor-related antigen to a sc MHC I molecule composed of β2 microglobulin and the three extracellular domains of the heavy chain of MHC I complexed with corresponding peptides (54). These virus- or tumor antigen-derived peptides can be loaded to the complexes as soluble peptides or can be genetically fused to the β2 microglobulin entity of the respective fusion proteins. In a proof-of-concept experiment, a sc HLA-A2 molecule, as described above, was genetically fused to a disulfide-bridged scFv domain of a humanized antibody directed against the interleukin 2 receptor α (IL2Rα) subunit, Tac (55) (Figure 1D). This fusion protein was produced in *E. coli* and functional proteins were generated by *in vitro* folding in the presence of HLA-A2 restricted peptides derived from the melanoma antigen gp100. Binding specificity was demonstrated by coating of Tac- expressing tumor cells with the fusion protein making them susceptible to specific lysis by HLA-A2-restricted gp100 peptide specific cytotoxic T-cells (CTLs). *In vivo* activity was demonstrated by monitoring the growth of the epidermoid carcinoma cell line ATAC4 in nude mice co-injected with specific CTLs (E:T ratio of 10:1) in the absence or presence of the fusion protein. In addition, these scAb-sc HLA-A2 complexes were further refined by fusing defined virus- or tumor antigen-derived peptides to the N-terminus *via* a flexible linker (56). These HLA-peptide complexes specifically activated CTLs directed against melanoma differentiation antigen gp100- or Epstein-Barr virus (EBV)-derived peptides. Similarly, scAb-sc HLA-A2 complexes targeted to the α-subunit of the IL2R or human mesothelin and loaded with peptides derived from melanoma differentiation antigen gp100 or EBV were evaluated *in vivo* after re-folding and loading with the corresponding peptide. *In vivo* efficacy was shown after intratumoral (*i.t.*) or intravenous (*i.v.*) injection of HLA-A2-restricted human CTLs for treatment of 40-50 mm^3 ATAC4 or A431/K5

xenograft tumors (57). In these experiments, 100 μg of fusion protein per mouse was administered daily and in several nude mice complete remissions were observed. The technology was also applied to epidermal growth factor receptor (EGFR)-expressing tumors. EGFR is an important mediator of tumor growth (58, 59) in many tumors and the anti-EGFR monoclonal antibody (mAb) cetuximab (C225) was approved for treatment of colorectal and head-and-neck cancer (60). A respective fusion protein consisting of sc HLA-A2 and scFv C225 was re-folded and loaded with peptides derived from gp100 or EBV (61). These fusion proteins were able to induce CTL-mediated lysis of EGFR-expressing tumor cells *in vitro* irrespective of the expression of peptide MHC on the tumor cells (61). In addition, the fusion proteins loaded with EBV-derived peptides were evaluated in Balb/c nude mice bearing established (50 mm^3) A-431-derived xenograft tumors. Fusion proteins (50 or 150 μg) were injected *i.v.* every other day for a total of three administrations, followed 6 h later by injection of 3×10^6 EBV-specific CTLs *i.v.* or *i.t.*. Remarkably, up to 94% reduction in tumor volume was observed. A crucial aspect for future development of the sc HLA-A2 fusion molecules will be the stabilization of the complexes by genetically fusing the corresponding peptides to the N-terminus of β2 microglobulin as shown for the prototype targeting of CD25 on leukemic cells (56).

TCR-like antibodies. Peptides derived from tumor-associated antigens are presented on the surface of tumor cells in the context of MHC I molecules. Thus, depending on the tumor-specific expression of these antigens, tumor-related pMHC complexes are created, which can serve as possible targets for antibody-derived therapeutic moieties (62-66). One such target class are differentiation antigens with cell lineage restricted expression. Examples are melanoma-specific proteins like tyrosinase-derived antigens, gp100 and MART-1 (67-69), which are expressed on melanocytes, or cancer testis antigens, such as NY-ESO-1, which are found in tumor cells apart from spermatogenic cells in the testis (70, 71). Another target class are proteins, which are mutated in cancer, such as ras, raf, p53 or β-catenin, or are over-expressed in cancer due to gene amplification or other mechanisms, such as human epidermal growth factor receptor 2 (HER2) (72-75).

Traditionally, new peptides have been identified by isolation of pMHC complexes from tumor-derived cell lines, elution of the MHC associated peptides and their subsequent identification by mass spectrometry (75). A new method for the identification of MHC-associated peptides makes use of the finding that in cell lines transfected with secreted HLA proteins these molecules are loaded with peptide and subsequently are secreted into the cell culture supernatant from which they can be purified. Peptides derived in such a way from tumor cell lines and corresponding normal cell lines can then be compared by mass spectrometry to identify specific tumor-associated peptide antigens (76, 77).

As a prerequisite for the generation of antibodies against pMHC complexes, procedures for the generation of their recombinant versions had to be established. This became possible by expressing the extracellular domains of HLA class I molecules and β2 microglobulin in *E. coli* as inclusion bodies and the subsequent refolding of those complexes in the presence of the corresponding peptides (78, 79). For the generation of pMHC specific antibodies, phage display libraries were an important tool to achieve this objective (80, 81). Thus, TCR-like antibodies were isolated from large libraries derived from naïve B-cells showing that the genetic information to generate these antibodies is present in the germline repertoire. *In vivo*, however, B-cells expressing these antibodies very likely are negatively selected (82, 83). In a similar approach, transgenic mice expressing an appropriate HLA molecule in the context of a murine MHC knock-out background were immunized with the desired pMHC complexes to establish a more focused library for a phage display approach (84, 85). Finally, standard hybridoma technology is also an option for generating TCR-like mabs. Originally, antigen-presenting cells expressing the appropriate HLA molecules and immunogenic peptides were used (86, 87). More recently, recombinantly-produced isolated MHC-peptide complexes were used as immunogens and high throughput screening was applied for the identification of hybridomas secreting TCR-like antibodies (88-90). In contrast to phage display-derived antibodies possessing affinities in the range of 50-300 nM, these hybridoma-derived TCR-like antibodies were reported to possess relatively high binding affinities in the low nanomolar range (1). One application of TCR-like antibodies is the quantification of specific pMHC complexes on the surface of tumor cells (91). In addition, cytotoxic properties can be conferred to TCR-like antibodies, *e.g.* by fusion with a truncated version of Pseudomonas exotoxin (92). The feasibility of such an approach was shown for molecules targeting melanoma antigens gp100 and MART-1 in the context of HLA-A2 (93), as well as for the breast and prostate antigen TCRγ alternative reading frame protein (TRAP) (94).

Several examples have demonstrated the potential of TCR-like antibodies to induce apoptosis specifically in tumor cells and to mediate ADCC and complement-dependent cytotoxicity (CDC) (62, 64, 76). Mimetic TCR (mTCR)-like antibodies were isolated and evaluated against various oncology-relevant target proteins, such as gp100, tyrosinase, MART-1 and -3 (melanoma), NY-ESO-1 (melanoma, breast, ovary and lung cancer), helicase p68 (breast cancer), HER2 (breast and ovarian cancer), TCR alternate reading frame protein 29 (TARP 29) (breast and prostate cancer), human chorionic gonadotropin β (hCGβ) (ovarian, breast, colon cancer), PR1, a peptide derived

from human proteinase 3 (leukemia), Wilms tumor antigen 1 (WT-1) (leukemia and other neoplasms), mucin 1 (MUC-1) (adenocarcinomas) and telomerase (multiple tumors) (61, 64, 76). In the following we highlight properties of mTCR-like antibodies directed against pMHC complexes specific for MUC-1, p68 helicase, migration inhibitory factor (MIF), hCGβ and HER2 (95-98). Thus, mAb RL-4B specific for MHC/hCGβ was efficacious *in vivo* in breast cancer xenograft models MDA-MB231 (ca. 4,000 pMHC complexes per cell) and MCF-7 (ca. 500 pMHC complexes per cell) after i.p. injection (95). These cell lines express the highest and lowest number of pMHC complexes per cell among all tumor cell lines investigated, respectively. Interestingly, *in vivo* efficacy was only partly dependent on Fc-mediated effector functions like ADCC and/or CDC since bivalent Fab (Fab)2 fragments were also able to mediate *in vivo* efficacy, albeit to a lower extent (96, 98). In this context, an effector cell independent mechanism of induction of apoptosis was identified, which involved activation and phosphorylation of mitogen-activated protein kinase (p38MAPK) and/or c-jun NH2-terminal kinase (jnk), activation of caspase 3 and poly ADP-ribosepolymerase 1 (PARP-1) (95-98). With regard to tumor cell specificity, mTCR RL21A, which specifically recognizes the MIF/HLA-A2 complex, detected invasive ductal carcinoma (IDC) but did not react with ductal carcinoma in situ, fibroadenoma, normal breast tissue, total white blood cells and a panel of normal tissues (97). The hCG-β/HLA-A2 specific antibody RL4B is specific for human primary breast tumor tissue but did not react, or reacted only weakly, with normal breast tissue from the same patient (95). Finally, mAb RL1B specific for HER2/HLA was able to suppress growth of HER2 low-expressing MDA-MB-231 xenograft tumors (98). Importantly, since this antibody does not interact with native HER2 it is also not adsorbed by shed HER2. From a regulatory point of view it should be stressed that the lack of a cross-reactive species for toxicity evaluation is a critical issue. Also, quantitation of pMHC complexes for specific targets is an unresolved issue, as well as the need for appropriate MHC I expression.

Chimeric antigen receptors (CARs) and T-cells redirected for universal cytokine signaling (TRUCKs). Based on encouraging results in malignant melanoma, adoptive immunotherapy, i.e. the reinfusion of cultivated tumor-infiltrating lymphocytes (TILs), has gained increasing attention (99). A further step was the modification and activation of T cells using chimeric antigen receptors (CARs), which are composed of an antigen-specific moiety and a module mediating T cell signaling. To generate CAR-bearing T cells, autologous T cells are transfected with expression constructs encoding the respective molecules and subsequently transfused into patients (100, 101). The efficacy of CARs was systematically improved by the introduction of protein domains, which are able to mediate co-stimulatory signals, and by adding domains that can trigger signaling pathways. This, in total, enhances proliferation, survival and cytokine production of T cells transfected with the respective expression constructs (102, 103). Additional therapeutic potential may be achieved by using T cells redirected for universal cytokine signaling (TRUCKs) (104). Here, the basic concept is to recruit a second wave of T cells in a locally restricted fashion to kill tumor cells, which are invisible to CARs, *e.g.* through down-regulation of the expression of antigens specifically recognized by CARs. Here, proof-of-concept experiments were performed with CAR expressing T cells producing IL12 conditionally following activation (105-107). IL12, in turn, is able to boost cytokine release, stimulate the growth of T cells and NK cells and revert the immune suppression induced by regulatory T cells, myeloid-derived suppressor cells (MDSCs), dendritic cells or macrophages (108). This local production of IL12 appears to be particularly relevant due to the toxicity of IL12 after systemic administration (109-112). Two clinical trials evaluating TRUCKs are underway (114). In addition, the use of CAR-modified T cells has recently received increased attention due to convincing clinical results in hematolological tumors (112-116). For example, CD19 is a suitable target for CAR-modified T cells because it is expressed exclusively on B-cells and B-cell-derived malignancies. Here, complete remissions were achieved in patients with anti-CD19 CARs. However, acute toxicities due to increased levels of inflammatory cytokines were noted (111, 112). In addition, depletion of endogenous lymphocytes by chemotherapy or radiotherapy before infusion of anti-CD19 CAR-transduced T cells was shown to enhance the *in vivo* activity of such T cells (112, 114, 116). The profound antigen-specific activity of anti-CD19 CAR-bearing T cells was further demonstrated by the long-term eradication of $CD19^+$ B-cells from treated patients (112). Importantly, especially robust and rapid anti-leukemic activity was demonstrated in heavily pretreated and chemotherapy refractory B-cell acute lymphocytic leukemia (B-ALL) patients using anti-CD19 CARs (116). Similarly, from 4 relapsed chronic lymphocytic leukemia (CLL) patients, who all received conditioning myeloablation therapy prior to T cell infusion, one patient had a complete response and the other three patients had partial responses (117). It will be interesting to see whether targets, such as CD138, CD56 and CD38 for multiple myeloma (118) and Le^y, CD33 and CD123 for acute myeloid leukemia (119-121), can be used for CAR-based therapies. Another pending issue is the optimization of CARs for successful treatment of solid tumors.

Conclusion

Certainly, cell-mediated immunity is central to anti-tumor responses exerted by the immune system, which can be induced *e.g.* by tumor-specific vaccination. However, functional and antigen specific pMHC/TCR interactions are an

indispensible prerequisite for the successful recognition of tumor cells by immune effector cells. Thus, for example, prophylactic vaccination against virus-induced tumors is approved for prevention of infection with human papilloma virus and, as a consequence, virus-induced cervical carcinoma (122). For therapeutic vaccination, many different strategies have been followed (123). For example, peptide cocktails for treatment of RCC have induced promising clinical responses (124). Another approach to generate long-lasting anti-tumoral responses was to revert the inhibition of tumor cell-specific T cells by using appropriate monoclonal antibodies (125). Thus, impressive clinical results have been obtained using monoclonal antibodies directed against cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed death receptor-1 (PD-1) and programmed death receptor-1 ligand (PD-1L) (126, 127). Hopefully, strategies for the induction of anti-tumor responses, as described in this review, will result in approved therapies for the treatment of cancer in the near future.

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