Published in final edited form as:

Expert Opin Biol Ther. 2016 August ; 16(8): 979–987. doi:10.1080/14712598.2016.1176138.

Opportunities and challenges for TCR mimic antibodies in cancer therapy

Aaron Y. Chang^{1,2}, Ron S. Gejman^{1,2}, Elliott J. Brea^{1,2}, Claire Y. Oh^{1,2}, Melissa D. Mathias¹, Dmitry Pankov¹, Emily Casey¹, Tao Dao¹, and David A. Scheinberg^{1,2,*}

¹Memorial Sloan Kettering Cancer Center, New York, New York, 10065

²Weill Cornell Medicine, New York, New York, 10065

Abstract

Introduction—Monoclonal antibodies (mAbs) are potent cancer therapeutic agents, but exclusively recognize cell-surface targets whereas most cancer-associated proteins are found intracellularly. Hence, potential cancer therapy targets such as overexpressed self-proteins, activated oncogenes, mutated tumor suppressors, and translocated gene products are not accessible to traditional mAb therapy. An emerging approach to target these epitopes is the use of TCR mimic mAbs (TCRm) that recognize epitopes similar to those of T cell receptors (TCR).

Areas covered—TCRm antigens are comprised of a linear peptide sequence derived from degraded proteins and presented in the context of cell-surface MHC molecules. We discuss how the nature of the TCRm epitopes provides both advantages (absolute tumor specificity and access to a new universe of important targets) and disadvantages (low density, MHC restriction, MHC down-regulation, and cross-reactive linear epitopes) to conventional mAb therapy. We will also discuss potential solutions to these obstacles.

Expert Opinion—TCRm combine the specificity of TCR recognition with the potency, pharmacologic properties, and versatility of mAbs. The structure and presentation of a TCRm epitope has important consequences related to the choice of targets, mAb design, available peptides and MHC subtype restrictions, possible cross-reactivity, and therapeutic activity.

Keywords

Monoclonal antibody; TCR mimic; TCR; oncofetal antigen; cancer-testis antigen; neoantigen; oncogene; phage display

1.0 Introduction

Most proteins in a cell are found intracellularly, including the proteins controlling cell growth, proliferation, and death. In a cancer cell, these proteins can include overexpressed oncogenic proteins, mutated tumor suppressors, and gene products of translocations. These intracellular cancer targets are not accessible to traditional monoclonal antibody (mAb) therapies. Furthermore, since many of these targets are not enzymes with readily druggable

*To whom correspondence should be addressed: scheinbd@mskcc.org, Phone: 646-888-2190, Fax: 646-422-0640.

pockets, these important oncogenic proteins cannot be easily inhibited with small molecules. Indeed, nearly all approved small molecule drugs are directed to a few percent of the available proteins within a cell. The remaining cancer-specific proteins, such as mutated oncogene products, transcription factors, protein adapters, and other nontraditional targets, remain inaccessible to current FDA-approved drugs. As a consequence, there are no truly tumor-specific therapeutic agents available for clinical use.

One approach to address these important and unique intracellular epitopes is to develop vaccines to these targets. Peptides derived from intracellular proteins can be presented on the cell surface in the context of major histocompatibility complex (MHC) class I, also referred to as human leukocyte antigen (HLA) class I, for recognition by T cell receptors (TCRs) on effector T cells (Figure 1).[1–3] An emerging approach that builds on this concept is the development of TCR mimic mAbs (TCRm) that recognize epitopes similar to those seen by a TCR.[4–7] These composite epitopes are comprised of linear peptide sequences bound to and presented by cell-surface MHC. Such epitopes can consist of peptides derived from overexpressed self-antigens and transcription factors, oncofetal antigens, cancer-testis antigens, translocation-derived amino acid sequences, viral neoantigens, or neoantigens created by the many mutations found in cancer. Because of the large number of proteins within cancer cells that fall into these and other categories, there are extensive opportunities for TCRm antibodies (Table 1). A recent report outlines several TCRm that have been developed against human epitopes found in various tumors.[8]

TCRm are structurally identical with traditional antibodies. However, while traditional antibodies recognize conformational epitopes, TCRm recognize a composite antigen comprised of a variable linear sequence (typically 9–10 amino acids in length) buried within an MHC molecule that is largely invariant.[1] The structure of this target has important consequences for antibody design, epitope restrictions, and possible cross-reactivities (Table 2). These issues will be discussed below.

The steps that lead from degradation of proteins into short peptides presented on MHC are well described. However, the precise rules that govern which protein fragments are ultimately presented on the cell surface are poorly understood and not fully predictable. Identification of presented epitopes is an empirical process. Some peptides may be generated but have low affinity to MHC. Other peptides may have high affinity to MHC, but never reach the cell surface due to improper processing.[9] Therefore, many potentially interesting targets are not available as MHC-presented cell-surface epitopes. Furthermore, HLA restriction selects small subsets of amino acid sequences for presentation, which means that an individual TCRm may work only for a subset of patients with particular HLA types.[10] Lastly, because the epitope consists of a linear peptide sequence within an MHC molecule (pMHC), there is also the possibility of cross-reactivity with other linear sequences that are homologous to the chosen epitope and may be presented on nontarget cells.

The unique features of TCRm distinguish them from other specific immunotherapies (Table 3). As discussed above, while TCRm display many of the drug-like properties of traditional mAbs, the nature of their target epitope provides both advantages and disadvantages. However, once a TCRm of appropriate specificity and affinity is developed, its

characteristics as an mAb make its clinical development relatively predictable and rapid. TCRm differ from TCR molecules because TCRm have predictable drug-like features and greater affinity (100–1000×) for targets.[11] TCRm also differ from cellular immunotherapies such as chimeric antigen receptor (CAR) T cells and adoptive T cells because they are not patient specific. CARs have demonstrated potent activity against hematological malignancies and patients have durable outcomes. [12–14] However, traditional CARs face the same limitations of traditional mAbs in that they are directed to cell surface antigens. In contrast, adoptive transfer of TCR-transduced T cells can be directed against an intracellular protein. This approach is in clinical trials and has the limitations of a patient-specific therapy. TCRs typically have substantially lower affinity than TCRm and may require re-engineering to improve their affinity. In addition, the transduced cells may lead to autoimmunity due to cross-activation of a previously tolerized T cell.[15] Therapeutic approaches that use living cells, such as CARs or adoptive T cells, cannot be easily controlled as to pharmacokinetics, level of therapeutic action at the tumor site, or duration of effects, and may lead to unacceptable toxicity, including death. Finally, the introduced TCR may dimerize with the endogenous TCR generating mismatched TCR with unknown specificity. Instead, TCRm constructs can be used to generate CARs that target intracellular proteins, offering an additional potent format. TCRm can be engineered (as with other antibodies) to enhance or delete their Fc region functionalities, alter their pharmacokinetics, or be re-engineered into bi-specific antibodies, bispecific T cell engagers (BiTEs), drug conjugates, radioconjugates, or CAR T cells.

However, as we will discuss, TCRm face challenges that traditional mAbs do not. The number of epitope sites for a TCRm that are presented on the cancer cell surface may be limited, yielding target densities orders of magnitude lower than typical targets of mAbs in clinical use. Low epitope density has important implications for potency, efficacy, and development of companion diagnostics for these therapeutic agents. In addition, specificity may be difficult to achieve with TCRm because some of the surface area of the TCRm Fab must be dedicated to recognizing the largely invariant MHC found on both on-and-off target pMHC.

2.0 Choosing the right antigen

Many of the caveats associated with the therapeutic efficacy of TCRm can be mitigated by choosing the correct target. (Table 1) TCRm can target intracellular proteins, including “undruggable” targets such as transcription factors, nuclear proteins, viral proteins, and other classes of oncoproteins.[16–19] TCRm can also react with pMHC derived from secreted proteins.[20] Identifying an ideal target for a TCRm requires consideration of the epitope abundance, presentation, specificity for cancer cells versus healthy cells, and heterogeneity of expression on tumor cells.

2.1 Differentiation antigens, cancer-testis antigens, and other tumor-associated antigens are valuable TCRm targets

Lineage or differentiation antigens presented by nonessential or medically replaceable organs, such as testes, ovary, prostate, endocrine glands, and subsets of leukocytes, are

potential TCRm targets. Traditional mAbs have often focused on these types of antigens and, while highly effective in many instances, still result in the killing of target-positive normal cells (e.g. anti-CD19 or CD20 antibodies used against B cell lymphomas). In clinical situations where the loss of a cell lineage is temporary, or where ameliorating treatment can be provided (e.g. intravenous immunoglobulins), this is a tolerable choice. Some solid tumors can also be approached in this way, such as prostate, ovary, and breast cancers in adults past childbearing age. In situations where lifelong function of the organ is more essential (e.g. thyroid and adrenal glands), organ ablation can be managed with appropriate hormone replacement. Several tumor-associated antigens exhibit tumor-restricted expression and are thus valuable TCRm targets. Cancer-testis antigens such as those from the MAGE gene family and NY-ESO1 are highly expressed in several tumor types but their expression in healthy adult tissue is restricted to the testes.[21] Oncofetal antigens such as Wilms' tumor 1 (WT1) and alpha-fetoprotein (AFP) are expressed during embryonic development and found in specific tumors, but are not expressed or have substantially limited expression in healthy adult tissues.[3,16] Furthermore, these tumor-associated antigens have been well-documented as targets for cytotoxic T lymphocytes (CTL) demonstrating proof of antigen presentation and validation of candidate targets.[16,22,23]

2.2 Neoantigens are simultaneously the most ideal and most difficult targets for TCRm

Mutated proteins are excellent targets for TCRm because they offer a high level of tumor specificity. However, they are also the most difficult to rationalize for therapeutic drug development given that few patients share the same neoantigens. Several groups have performed whole-exome sequencing of tumors, identified candidate neoantigens *in silico*, verified HLA binding *in vitro*, and even identified tumor infiltrating lymphocytes specific to these mutant epitopes.[24] Unfortunately, the majority of tumor mutations in carcinomas and other heavily mutated cancers are not shared between patients. Therefore, designing TCRm antibodies to these private neoantigens as a broad strategy to treat cancer is prohibitively costly using current technology.

On the other hand, early work has identified recurrent mutations (>1% of patients) that are predicted to result in neoantigens.[25] Research groups designing TCRm antibodies should focus on identifying recurrent neoantigens (such as those found in KRAS G12V/C, p53,[26] or BCR-ABL).[27,28] Despite the low likelihood of a tumor having any given neoantigen, a sufficiently large population may benefit from the development of high-quality TCRm to neoantigens with as little as 1% tumor prevalence.

Databases such as the Cancer Genome Atlas can be utilized to identify mutated protein sequences as targets.[29] While these databases can serve as useful starting points, direct evidence of antigen presentation from mass spectrometry or evidence of T-cell killing of pMHC-presenting tumor cells *in vitro* is necessary. Unfortunately, current methods to identify peptides presented by cancer cells on surface MHC may lack the required sensitivity and thus identify only a subset of possible antigens.[1] Therefore, epitopes presented at low levels, such as those likely to be TCRm targets, may not be detected.

Given all of these obstacles, more comprehensive strategies for identifying MHC antigens must be developed by using prediction algorithms based on RNA-seq data and mass spectrometry of peptides.

To date, nearly all efforts to produce TCRm have been developed for MHC class I epitopes, largely because the tools and reagents directed to these molecules are now widely available. In addition, the prediction algorithms *in silico* for identifying epitopes are more accurate for MHC class I epitopes. Furthermore, the promiscuity of the types of sequences found in MHC class II epitopes may complicate the specificity analyses. Despite these limitations, it is important that TCRm to class II antigens be developed in the future. As an example, the antibody clone G3H8 was designed to recognize and block a type I diabetes MHC class II autoantigen. [30] G3H8 recognizes GAD^{555–567} in the context of HLA-DR4 on antigen presenting cells and inhibits HLA-DR4-restricted and GAD^{555–567}-specific T cell responses. [30] Such studies demonstrate that TCRm may have utility in modulating CD4 T cell responses in autoimmune and other diseases.

2.3 TCRm may bind to off-target sequences that limit their applicability

Several TCRm have now been described and it is possible to identify some general trends in these molecules that distinguish them from traditional mAbs. Traditional mAbs bind to conformational epitopes. In contrast, TCRm recognize a complex antigen consisting of an MHC molecule with a short linear sequence embedded within it. The MHC component is invariant for a given haplotype, while the embedded epitope can be derived from millions of sequences encoded in the exome. Most TCRm have been shown to bind only a few residues of their target linear peptide. [5,16–18] This suggests that the TCRm, in theory, could have many off-target epitopes that share the same residues at major contact positions, but differ at other positions. For example, the therapeutic TCRm ESK1 binds to a composite antigen composed of HLA-A*02:01 amino acids in addition to three to five N-terminal residues of a WT1-derived peptide 9-mer.[10,16] Substitutions of the C-terminal amino acids of the target peptide still allows binding of the ESK1 TCRm. In addition, another TCRm (named 8F4) against the tumor-associated antigen PR1 has been shown to depend heavily on one residue of the PR1-peptide,[17] whereas a TCRm to the cancer-testis antigen PRAME[31] was shown to bind to the C-terminal end of the peptide sequence. Homologous peptide sequences may exist in the exome which yield cross-reactive epitopes on healthy cells, thereby reducing TCRm specificity. However, it is important to note that potential off-target peptide sequences may not be correctly processed and presented onto cell-surface MHC.

3.0 Enhancing epitope density to increase potential of TCRm

Presentation of a peptide on MHC requires sufficient intracellular expression of the parent protein, proper proteolytic cleavage, peptide transport into the endoplasmic reticulum (ER) via the TAP proteins, and efficient loading of the peptide onto MHC molecules for shuttling to the cell surface. Currently, these processes are difficult to model *in silico*, making it challenging to predict the degree of cell-surface expression for a given epitope. pMHC presentation has been demonstrated to be directly related to various factors including the level of protein expression and rate of protein degradation.[1] Therefore, strategies to

understand, predict, and enhance epitope presentation are crucial. Pharmacological modulation of these processes may enhance TCRm therapy by increasing epitope presentation.

3.1 Epigenetic regulation

Tumor-associated antigens often originate from genes that are under epigenetic regulation. Thus, targeting chromatin-remodeling enzymes can augment target protein expression. For example, the hypomethylating agent decitabine can dramatically increase expression of NY-ESO1 and MAGE-3 in patient tumor biopsies.[32] Another methyltransferase inhibitor, 5-azacytidine, can also induce cancer-testis antigen-specific CTLs in patients while minimally affecting immune effector populations and functions.[33] This suggests that epigenetic drugs can drive presentation of tumor antigens by enhancing protein expression without substantially attenuating immune effector recruitment and cytotoxicity.

3.2 Protein processing and presentation

Protein processing machinery is important in driving presentation of a TCRm epitope. The proteasome is the major complex that degrades proteins into peptides and exists in two major forms: the constitutive proteasome (CP) or the immunoproteasome (IP). Compared to the CP, the IP has altered cleavage specificities, is mainly found in leukocytes, and can be induced in other cells upon treatment with pro-inflammatory cytokines such as IFN γ and TNF α . Peptides can be restricted to the CP or IP. Hence, modulating the expression of the proteasome forms through cytokine treatment can enhance or abrogate presentation of a specific peptide.[34] Peptides that are generated by both the CP and IP thus represent desired targets due to more global presentation. In addition, proteasome inhibitors are being evaluated as anti-tumor therapies. Counter-intuitively, proteasome inhibitors can augment presentation of specific peptides by shifting the catalytic specificities. For example, the tumor antigen MAGE-3^{271–279} is only substantially presented by melanoma cells after proteasome inhibition by lactacystin.[22] Specific proteasomal cleavages can either generate or destroy an immunogenic peptide via an internal digest, such as the influenza M1^{58–66} peptide, which is destroyed by the proteasome, but more efficiently generated in the presence of lactacystin.[9] Therefore, proteasome inhibitors shift the proteasome cleavage specificities, alter the repertoire of proteasome-generated peptides, and can either increase or decrease target epitope presentation. More accurate tools to predict proteasome cleavage sites will help to identify promising TCRm targets.

After degradation, the cytosolic peptides are pumped into the ER lumen by TAP1 and TAP2, where the N-terminus can be trimmed by aminopeptidases before binding MHC class I. Down-regulation of the antigen processing machinery prevents even a properly processed peptide from reaching the surface.

A serious obstacle to TCRm therapy involves decreased MHC class I expression on the cancer cell surface. Although this event is highly variable, up to 90% MHC class I down-regulation has been noted in some cancers.[35] Epigenetic alterations in cancer have been linked to decreased transcription of MHC.[36] This may be reversed using agents that inhibit histone deacetylation or induce DNA hypomethylation which has been shown to increase

MHC expression.[36] Moreover, inhibition of the MAPK pathway with the MEK inhibitor PD98059 has been shown to increase MHC expression in esophageal and gastric cancers. [37] Biopsies of patients treated with the EGFR inhibitor erlotinib show increased MHC class I levels and cells treated with the EGFR antibody cetuximab demonstrate enhanced target cell recognition by CTLs.[38,39] Another level of regulation involves targeting beta-2-microglobulin (β 2M), which is needed for MHC class I presentation. β 2M is often downregulated in cancers, suggesting that modulators of β 2M may increase target epitope presentation.[40]

Several pharmacological targets discussed above are already used clinically and have demonstrated anti-tumor efficacy through mechanisms independent of TCRm. Therefore, using these agents to increase target epitope presentation in combination with TCRm therapy may be synergistic and prevent resistance to monotherapy.

4.0 Methods to enhance potency of TCRm may be needed

The cell-surface density of a TCRm epitope is significantly lower than that of current commercially available therapeutic mAbs, which may limit TCRm therapeutic activity. A strategy to overcome the potential hurdle of low epitope density is to enhance TCRm potency. TCRm are IgGs and therefore the same strategies to enhance therapeutic efficacy of traditional mAb therapies can thus be applied to TCRm.

Mechanisms of action of mAbs include antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP) mediated by immune effector cells (e.g. NK cells, monocytes/macrophages, and neutrophils), complement-dependent cytotoxicity (CDC), and Fc-independent direct cytotoxicity (e.g. blocking of oncogenic or growth factor signaling). All of these functions are epitope density-dependent and perhaps epitope geometry-dependent,[41] but the degree to which each mechanism is regulated by these factors is unclear. CDC has been observed with some TCRm, such as 8F4 which recognizes the PR1 antigen,[17] while other have demonstrated direct cytotoxic effects.[42] However, these mechanisms are not widely observed with the majority of current TCRm.

Enhancing ADCC/ADCP has been widely used to improve the therapeutic efficacy of many mAbs.[43] The first human TCRm specific for the WT1-derived peptide, RMFPNAPYL, in the context of HLA-A*02:01 molecule, ESK1, was modified to create an enhanced Fc γ receptor (Fc γ R) binding version of the mAb. This TCRm, called ESKM, was engineered to yield an afucosylated chain at Asn297 within the Fc region, therefore exposing a terminal mannose.[16,44–46] ESKM showed increased affinity for activating Fc γ Rs (mouse Fc γ RIV and human Fc γ RIIIa) as well as decreased affinity for the inhibitory Fc γ RIIb receptor, leading to enhanced ADCC and therapeutic efficacy in both *in vitro* and animal studies.[45] Increased Fc γ R binding on macrophages also enhance ADCP.[47] Enhanced ADCP may also be achieved by combining TCRm with mAbs that release the inhibitory signals controlling macrophage phagocytosis, such the anti-CD47 mAb. CD47 is a “do not eat me” signaling molecule that is up-regulated in many cancer cells, therefore reducing macrophage phagocytosis.[48] In mouse models of non-Hodgkin lymphoma, combining anti-CD47 mAb with rituximab has been shown to be superior to single mAb therapy.[49] Combination

therapy with other drugs could also improve therapeutic efficacy as has been shown with combination therapy of the ESKM mAb and a variety of tyrosine kinase inhibitors for the treatment of CML.[44]

Engineering bispecific mAbs and bispecific T cell engagers (BiTEs) to bring in powerful polyclonal T cell cytotoxicity has been shown to be an effective therapeutic strategy in clinical use. BiTEs are heterodimers of single chain variable fragments (scFvs) derived from two distinct antibodies. They are designed to cross-link a particular surface antigen on cancer cells to the TCR/CD3 complex on T cells. BiTE molecules can redirect both CD4 and CD8 T cells to kill tumor cells in a serial fashion that is independent of the cells' intrinsic antigen specific TCR recognition or co-stimulatory molecules.[50] One concern about this approach is whether the monovalent scFv construct is able to selectively and adequately bind low-density pMHC complexes on the cell surface due to the reduced binding affinity as compared to the bivalent TCRm. Despite these concerns, the WT1 peptide-specific ESK-BiTE, a BiTE construct derived from the ESK1 scFv, was able to selectively bind WT1/HLA-A*02:01 positive tumor cells and showed potent therapeutic activity *in vitro* and *in vivo* against multiple human cancer models by redirecting human T cell cytotoxicity.[46] Interestingly, in addition to its immediate cytotoxicity against tumor cells, the ESK-BiTE induced epitope-spreading, which could contribute to the potent and long-term therapeutic activity of this BiTE construct.[46]

A related concept for redirecting polyclonal T cell cytotoxicity has also been explored by engineering chimeric antigen receptor (CAR) T cells. CAR constructs link the scFv of a mAb specific for the target antigen to the intracellular CD3 zeta chain, along with a costimulatory domain, such as CD28, 4-1BB, and possibly cytokine IL-12 expression in order to enhance T cell activation and survival. CAR constructs are transduced into patient's T cells, which are in turn expanded *in vitro* and re-infused back into patients.[51] TCRm can also be used to create CAR T cells such as CARs generated with TCRm against a WT1 peptide and the CAR generated with the TCRm GPA7 to target a gp100 peptide in melanoma. [52–54] Such TCRm CARs demonstrated the ability to redirect T cell cytotoxicity against tumors and the GPA7 CAR suppressed melanoma progression in a xenograft model. [53]

Another well-studied and validated approach to enhance potency of classical mAbs is to conjugate the antibody to cytotoxic agents, such as radioisotopes, cytotoxic drugs, and toxins. Antibody drug conjugates (ADCs) combine the specificity of a monoclonal antibody to a highly potent cytotoxic drug. Analogously, a radioconjugate delivers a radioactive element, typically emitting a short-range alpha particle or beta particle to the surface or inside the target cell. In doing so, they lead to directed tumor killing, reduced systemic toxicity, and altered pharmacokinetics of the cytotoxic agent. Several ADC and radioconjugates are already marketed. TCRm Fabs specific for melanoma peptides derived from MART-1 and gp100 presented on HLA-A*02:01 have been conjugated to a truncated form of *Pseudomonas* exotoxin and have successfully inhibited melanoma cell growth both *in vitro* and *in vivo*. [55] The ADC approach requires internalization of the mAb/antigen complex, which may not be efficient for MHC bound antibodies. For a mAb with poor internalization,

conjugating the mAb to short-ranged, highly cytotoxic alpha particle emitters could bypass this problem as they can kill cells without internalization.[56]

5.0 Conclusion

The large number of therapeutic successes achieved by mAbs selective for cancer and the vast new array of potential targets afforded by TCRm suggests that this emerging class of cancer therapeutic agents will find an important place in our armamentarium. Historically, only CTLs could reach important intracellular tumor-specific antigens. However, TCRm combine the true cancer specificity of TCR recognition with the potency, drug-like properties, and versatility of mAb. The advantages of mAb-based therapy include their high target specificity, high efficacy, predictable and limited side effects, long plasma half-life, and infrequent dosing. TCRm-based agents avoid the disadvantages of patient-specific adoptive T cell and CAR T cell-based therapies, which require *ex vivo* expansion and re-infusion, but can still achieve the potency of a redirected T cell effector. In this way TCRm antibodies represent an ideal next step for immunotherapy by targeting intracellular tumor antigens.

6.0 Expert Opinion

TCRm mAbs change the paradigm for the targets that are accessible to antibodies. The studies *in vivo* discussed above demonstrate their therapeutic efficacy. However, it is important to understand the challenges for TCRm compared to traditional mAbs and possible methods to mitigate these limitations before moving TCRm into clinical trials.

Tumor specificity is a major hurdle in developing effective TCRm. Ideal targets are derived from proteins that have little or no expression in normal tissue yet generate enough tumor cell-surface presentation to allow TCRm-mediated cytotoxicity *in vivo*. Identifying truly tumor-specific antigens is a major challenge (Table 1). One approach is to target viral proteins expressed in virally transformed cancer cells. Viral RNA is present in large proportions of some cancers, such as cervical cancer and head and neck cancer.[57,58] Some of these oncogenic viruses produce validated HLA binding epitopes, such as the E6 protein of HPV-16.[2] Another approach is to target recurrent oncogenic fusion proteins formed by chromosome translocations, such as t(9:22) BCR-ABL,[28,59] or point mutations unique to an oncogenic protein or tumor suppressor such as Ras or p53.

A major weakness of currently investigated TCRm is that binding to pMHC rely on only a few contact residues on the peptide, suggesting possible cross-reactivity with off-target partly homologous peptide sequences. Candidate TCRm clones are typically screened using an assay that is based on density or affinity to a pMHC of interest until a single clone is picked to undergo more comprehensive specificity and therapeutic efficacy testing. One possibility is that the initial selection methods are biasing chosen TCRm to those clones that bind in large numbers to target cells—at the expense of high specificity. This may lead to selecting clones that have higher cross-reactivity with homologous epitopes—or to the invariant MHC. We propose that investigators making TCRm by phage display use appropriate libraries of irrelevant pMHC to negatively select phage clones during panning.

Such libraries may ensure that TCRm-pMHC contacts are not selected on 1–2 target residue interactions, but instead form broad contacts across the whole pMHC. In addition, better methods for validating the specificity of TCRm are needed. Off-targets of TCRm are currently of unclear clinical significance, but should be addressed before these agents enter the clinic. Mutational scanning (e.g. alanine scanning) is the principal tool used to identify potential off-targets of TCRm. This low-throughput technique is not sufficient to identify the millions of possible HLA-binding epitopes. Instead, high-throughput methods to screen TCRm against large libraries of HLA-binding ligands are needed. More crystal structures of TCRm bound to their epitopes will aid in developing the best methods and better *in silico* algorithms for ensuring specificity.

A major strength of TCRm is that the mAb is a well-characterized drug format that can be engineered for augmented potency, including CAR T cells or bispecific antibodies. We believe that Fc engineering and the BiTE format will be instrumental to enhance TCRm potency against a low density pMHC target. The epitope spreading induced by the ESK-BiTE construct discussed also has unique implications for TCRm-BiTE mechanism of cytotoxicity. We expect that such epitope spreading might contribute to therapeutic efficacy by preventing outgrowth of tumor cells that do not express the target protein at required levels. Eliciting a secondary response against several unrelated pMHC epitopes could also prevent resistance associated with down-regulation of the primary target.

Inadequate antigen presentation is another important hurdle. If MHC expression or the required antigen processing machinery is down-regulated, we propose evaluating combination therapy with discussed pharmacological agents such as histone deacetylase inhibitors, proteasome inhibitors, and MEK inhibitors. Therefore, combining strategies to achieve definitive specificity and maximum potency will generate TCRm antibodies with powerful potential in cancer immunotherapy.

References

References were chosen because they were the first demonstration of a new concept or the best example of that concept.

1. Bassani-Sternberg M, Pletscher-Frankild S, Jensen LJ, Mann M. Mass spectrometry of human leukocyte antigen class I peptidomes reveals strong effects of protein abundance and turnover on antigen presentation. *Mol Cell Proteomics*. 2015; 14(3):658–673. [PubMed: 25576301]
Demonstration of current capability and limitations using mass spectrometry to determine MHC-presented peptides
2. Draper LM, Kwong MLM, Gros A, et al. Targeting of HPV-16+ epithelial cancer cells by TCR gene engineered T cells directed against E6. *Clin Cancer Res*. 2015; 21(19):4431–4439. [PubMed: 26429982]
3. Mizukoshi E, Nakamoto Y, Tsuji H, Yamashita T, Kaneko S. Identification of alpha-fetoprotein-derived peptides recognized by cytotoxic T lymphocytes in HLA-A24+ patients with hepatocellular carcinoma. *Int J Cancer*. 2006; 118(5):1194–1204. [PubMed: 16152611]
4. Wittman VP, Woodburn D, Nguyen T, Neethling FA, Wright S, Weidanz JA. Antibody Targeting to a Class I MHC-Peptide Epitope Promotes Tumor Cell Death. *J Immunol*. 2006; 177(6):4187–4195. [PubMed: 16951384]
5. Polakova K, Plaksin D, Chung DH, Belyakov IM, Berzofsky JA, Margulies DH. Antibodies Directed Against the MHC-I Molecule H-2Dd Complexed with an Antigenic Peptide: Similarities to

- a T Cell Receptor with the Same Specificity. *J Immunol.* 2000; 165(10):5703–5712. [PubMed: 11067928] An early description of the approach for mAb with TCR-like features
6. Lev A, Denkberg G, Cohen CJ, et al. Isolation and characterization of human recombinant antibodies endowed with the antigen-specific, major histocompatibility complex-restricted specificity of T cells directed toward the widely expressed tumor T-cell epitopes of the telomerase catalytic sub. *Cancer Res.* 2002; 62(11):3184–3194. [PubMed: 12036932]
 7. Denkberg G, Cohen CJ, Lev A, Chames P, Hoogenboom HR, Reiter Y. Direct visualization of distinct T cell epitopes derived from a melanoma tumor-associated antigen by using human recombinant antibodies with MHC-restricted T cell receptor-like specificity. *Proc Natl AcadSci U S A.* 2002; 99(14):9421–9426.
 8. Dahan R, Reiter Y. T-cell-receptor-like antibodies - generation, function and applications. *Expert Rev Mol Med.* 2012; 14(February):e6. [PubMed: 22361332]
 9. Luckey CJ, King GM, Marto JA, et al. Proteasomes Can Either Generate or Destroy MHC Class I Epitopes: Evidence for Nonproteasomal Epitope Generation in the Cytosol. *J Immunol.* 1998; 161:112–121. [PubMed: 9647214]
 10. Ataie N, Xiang J, Cheng N, et al. Structure of a TCR mimic antibody with target predicts pharmacogenetics. *J Mol Biol.* 2015 Dec.
 11. Stewart-jones G, Hombach A, Shenderov E, et al. Rational development of high-affinity T-cell receptor-like antibodies. *Proc Natl Acad Sci.* 2009; 106(26):10872–10872. Comparison of the affinity and peptide contacts between a TCR and TCRm Fab against an epitope of NY-ESO1
 12. Gill S, June CH. Going viral: chimeric antigen receptor T-cell therapy for hematological malignancies. *Immunol Rev.* 2015; 263(1):68–89. [PubMed: 25510272]
 13. Curran KJ, Brentjens RJ. Chimeric Antigen Receptor T Cells for Cancer Immunotherapy. *J ClinOncol.* 2015; 33(15):1703–1706.
 14. Brentjens RJ, Davila ML, Riviere I, et al. CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. *Sci Transl Med.* 2013; 5(177):177ra38.
 15. Starck L, Popp K, Pircher H, Uckert W. Immunotherapy with TCR-Redirected T Cells: Comparison of TCR-Transduced and TCR-Engineered Hematopoietic Stem Cell-Derived T Cells. *J Immunol.* 2014; 192(1):206–213. [PubMed: 24293634]
 16. Dao T, Yan S, Veomett N, et al. Targeting the intracellular WT1 oncogene product with a therapeutic human antibody. *Sci Transl Med.* 2013; 5(176) Proof that TCRm can be therapeutically active in animal models
 17. Sergeeva A, Alatrash G, He H, et al. An anti-PR1/HLA-A2 T-cell receptor-like antibody mediates complement-dependent cytotoxicity against acute myeloid leukemia progenitor cells. *Blood.* 2011; 117(16):4262–4272. [PubMed: 21296998] Demonstration of TCRm-mediated cytotoxicity against a tumor-associated differentiation antigen
 18. Sastry KSR, Too CT, Kaur K, et al. Targeting hepatitis B virus-infected cells with a T-cell receptor-like antibody. *J Virol.* 2011; 85(5):1935–1942. [PubMed: 21159876]
 19. Kim S, Pinto AK, Myers NB, et al. A novel T-cell receptor mimic defines dendritic cells that present an immunodominant West Nile virus epitope in mice. *Eur J Immunol.* 2014; 44(7):1936–1946. [PubMed: 24723377]
 20. Correale P, Walmsley K, Nieroda C, et al. In vitro generation of human cytotoxic T lymphocytes specific for peptides derived from prostate-specific antigen. *J Natl Cancer Inst.* 1997; 89(4):293–300. [PubMed: 9048833]
 21. Almstedt M, Blagitko-Dorfs N, Duque-Afonso J, et al. The DNA demethylating agent 5-aza-2'-deoxycytidine induces expression of NY-ESO-1 and other cancer/testis antigens in myeloid leukemia cells. *Leuk Res.* 2010; 34(7):899–905. [PubMed: 20381863]
 22. Valmori BD, Gileadi U, Servis C, et al. Modulation of Proteasomal Activity Required for the Generation of a Cytotoxic T Lymphocyte – defined Peptide Derived from the Tumor Antigen MAGE-3. *J Exp Med.* 1999; 189(6)
 23. Michaeli Y, Denkberg G, Sinik K, et al. Expression hierarchy of T cell epitopes from melanoma differentiation antigens: unexpected high level presentation of tyrosinase-HLA-A2 Complexes

- revealed by peptide-specific, MHC-restricted, TCR-like antibodies. *J Immunol.* 2009; 182(10): 6328–6341. [PubMed: 19414786]
24. Tran E, Ahmadzadeh M, Lu Y, et al. Immunogenicity of somatic mutations in human gastrointestinal cancers. *Science* (80-). 2015; 350(6266):1387–1390.
 25. Karasaki T, Nagayama K, Kawashima M, et al. Identification of Individual Cancer-Specific Somatic Mutations for Neoantigen-Based Immunotherapy of Lung Cancer. *J Thorac Oncol.* 2015 Identification of recurrent neoantigens in lung cancer as potential tumor-specific targets
 26. Ito D, Visus C, Hoffmann TK, et al. Immunological characterization of missense mutations occurring within cytotoxic T cell-defined p53 epitopes in HLA-A*0201+ squamous cell carcinomas of the head and neck. *Int J Cancer.* 2007; 120(12):2618–2624. [PubMed: 17294448]
 27. Cai A, Keskin DB, DeLuca DS, et al. Mutated BCR-ABL generates immunogenic T-cell epitopes in CML patients. *Clin Cancer Res.* 2012; 18(20):5761–5772. [PubMed: 22912393]
 28. Bocchia M, Korontsvit T, Xu Q, et al. Specific human cellular immunity to bcr-abl oncogene-derived peptides. *Blood.* 1996; 87(9):3587–3592. [PubMed: 8611681]
 29. Chang K, Creighton CJ, Davis C, et al. The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet.* 2013; 45(10):1113–1120. [PubMed: 24071849]
 30. Dahan R, Gebe JA, Preisinger A, et al. Antigen-specific immunomodulation for type 1 diabetes by novel recombinant antibodies directed against diabetes-associated auto-reactive T cell epitope. *J Autoimmun.* 2013; 47:83–93. [PubMed: 24090977]
 31. Chang, AY.; Dao, T.; Scott, A., et al. A Therapeutic TCR Mimic Monoclonal Antibody for Intracellular PRAME Protein in Leukemias. Poster Presentation: 57th American Society of Hematologists Annual Meeting; December 2015; Orlando, FL.
 32. Schrump DS, Fischette MR, Nguyen DM, et al. Phase I study of decitabine-mediated gene expression in patients with cancers involving the lungs, esophagus, or pleura. *Clin Cancer Res.* 2006; 12(19):5777–5785. [PubMed: 17020984]
 33. Gang AO, Frøsig TM, Brimnes MK, et al. 5-Azacytidine treatment sensitizes tumor cells to T-cell mediated cytotoxicity and modulates NK cells in patients with myeloid malignancies. *Blood Cancer J.* 2014 Feb.4:e197. [PubMed: 24681961]
 34. Ma W, Vigneron N, Chapiro J, et al. A MAGE-C2 antigenic peptide processed by the immunoproteasome is recognized by cytolytic T cells isolated from a melanoma patient after successful immunotherapy. *Int J Cancer.* 2011; 129(10):2427–2434. [PubMed: 21207413]
 35. Cabrera T, Angustias Fernandez M, Sierra A, et al. High frequency of altered HLA class I phenotypes in invasive breast carcinomas. *Hum Immunol.* 1996; 50(2):127–134. [PubMed: 8891736]
 36. Campoli M, Ferrone S. HLA antigen changes in malignant cells: epigenetic mechanisms and biologic significance. *Oncogene.* 2008; 27(45):5869–5885. [PubMed: 18836468]
 37. Mimura K, Shiraishi K, Mueller A, et al. The MAPK pathway is a predominant regulator of HLA-A expression in esophageal and gastric cancer. *J Immunol.* 2013; 191(12):6261–6272. [PubMed: 24244023]
 38. Pollack BP, Sapkota B, Cartee T V. Epidermal growth factor receptor inhibition augments the expression of MHC class I and II genes. *Clin Cancer Res.* 2011; 17(13):4400–4413. [PubMed: 21586626]
 39. Srivastava RM, Trivedi S, Concha-Benavente F, et al. STAT1-Induced HLA Class I Upregulation Enhances Immunogenicity and Clinical Response to Anti-EGFR mAbCetuximab Therapy in HNC Patients. *Cancer Immunol Res.* 2015:1–11. [PubMed: 25568067]
 40. Palmisano GL, Pistillo MP, Capanni P, et al. Investigation of HLA class I downregulation in breast cancer by RT-PCR. *Hum Immunol.* 2001; 62(2):133–139. doi:S0198-8859(00)00241-X [pii]. [PubMed: 11182222]
 41. Diebold, Ca; Beurskens, FJ.; de Jong, RN., et al. Complement is activated by IgG hexamers assembled at the cell surface. *Science.* 2014 Mar.343:1260–1263. [PubMed: 24626930]
 42. Verma B, Neethling FA, Caseltine S, et al. TCR mimic monoclonal antibody targets a specific peptide/HLA class I complex and significantly impedes tumor growth in vivo using breast cancer models. *J Immunol.* 2010; 184(4):2156–2165. [PubMed: 20065111]

43. Hayes JM, Cosgrave EF, Struwe WB, et al. Glycosylation and Fc Receptors. *Curr Top Microbial Immunol.* 2014; 382:165–199.
44. Dubrovsky L, Pankov D, Brea EJ, et al. A TCR-mimic antibody to WT1 bypasses tyrosine kinase inhibitor resistance in human BCR-ABL+ leukemias. *Blood.* 2014; 123(21):3296–3304. [PubMed: 24723681]
45. Veomett N, Dao T, Liu H, et al. Therapeutic efficacy of an Fc-enhanced TCR-like antibody to the intracellular WT1 oncoprotein. *Clin Cancer Res.* 2014; 20(15):4036–4046. [PubMed: 24850840]
46. Dao T, Pankov D, Scott A, et al. Therapeutic bispecific T-cell engager antibody targeting the intracellular oncoprotein WT1. *Nat Biotechnol.* 2015 Sep.33:1–10. [PubMed: 25574611] TCRm engineered into a bispecific T-cell engager format can induce epitope spreading
47. Jung ST, Kelton W, Kang TH, et al. Effective Phagocytosis of Low Her2 Tumor Cell Lines with Engineered, Aglycosylated IgG Displaying High FcγRIIa Affinity and Selectivity. *ACS Chem Biol.* 2013; 8(2):368–375. [PubMed: 23030766]
48. McCracken MN, Cha AC, Weissman IL. Molecular pathways: Activating T cells after cancer cell phagocytosis from blockade of CD47 “Don’t eat Me” signals. *Clin Cancer Res.* 2015; 21(16): 3597–3601. [PubMed: 26116271]
49. Chao MP, Alizadeh AA, Tang C, et al. Anti-CD47 Antibody Synergizes with Rituximab to Promote Phagocytosis and Eradicate Non-Hodgkin Lymphoma. *Cell.* 2010; 142(5):699–713. [PubMed: 20813259]
50. Nagorsen D, Baeuerle PA. Immunomodulatory therapy of cancer with T cell-engaging BiTE antibody blinatumomab. *Exp Cell Res.* 2011; 317(9):1255–1260. [PubMed: 21419116]
51. Curran KJ, Pegram HJ, Brentjens RJ. Chimeric antigen receptors for T cell immunotherapy: current understanding and future directions. *J Gene Med.* 2012; 14(6):405–415. [PubMed: 22262649]
52. Rafiq, S.; Dao, T.; Liu, C., et al. Engineered T Cell Receptor-Mimic Antibody, (TCRm) Chimeric Antigen Receptor (CAR) T Cells Against the Intracellular Protein Wilms Tumor-1 (WT1) for Treatment of Hematologic and Solid Cancers. Poster Presentation 56th American Society of Hematologists Annual Meeting; December 2014; San Francisco, CA.
53. Zhang G, Wang L, Cui H, et al. Anti-melanoma activity of T cells redirected with a TCR-like chimeric antigen receptor. *Sci Rep.* 2014; 4:3571. [PubMed: 24389689] TCRm can be engineered into CAR T cells to direct cellular immunotherapy against intracellular proteins
54. Oren R, Hod-Marco M, Haus-Cohen M, et al. Functional comparison of engineered T cells carrying a native TCR versus TCR-like antibody-based chimeric antigen receptors indicates affinity/avidity thresholds. *J Immunol.* 2014; 193(11):5733–5743. [PubMed: 25362181]
55. Klechevsky E, Gallegos M, Denkberg G, et al. Antitumor activity of immunotoxins with T-cell receptor-like specificity against human melanoma xenografts. *Cancer Res.* 2008; 68(15):6360–6367. [PubMed: 18676861]
56. McDevitt MR, Ma D, Lai LTL, et al. Tumor therapy with targeted atomic nanogenerators. *Science* (80-). 2001; 294(5546):1537–1540.
57. Tang K-W, Alaei-Mahabadi B, Samuelsson T, Lindh M, Larsson E. The landscape of viral expression and host gene fusion and adaptation in human cancer. *Nat Commun.* 2013; 4:2513. [PubMed: 24085110]
58. Makler O, Oved K, Netzer N, Wolf D, Reiter Y. Direct visualization of the dynamics of antigen presentation in human cells infected with cytomegalovirus revealed by antibodies mimicking TCR specificity. *Eur J Immunol.* 2010; 40(6):1552–1565. [PubMed: 20306470]
59. Worley BS, van den Broeke LT, Goletz TJ, et al. Antigenicity of Fusion Proteins from Sarcoma-associated Chromosomal Translocations. *Cancer Res.* 2001; 61(18):6868–6875. [PubMed: 11559563]

Article Highlights

- Traditional monoclonal antibodies (mAbs) exclusively recognize cell-surface targets.
- Most cancer-specific targets are not accessible to traditional mAb therapy.
- TCR mimic mAbs (TCRm) recognize epitopes similar to those of T cell receptors (TCR) derived from proteins inside the cell.
- TCRm combine the specificity of TCR recognition with the potency, pharmacologic properties, and versatility of mAbs.
- TCRm epitopes provide advantages in tumor specificity and access to a new universe of important targets.
- However, TCRm epitopes are characterized by low density, MHC restriction, potential MHC down-regulation, and cross-reactive linear epitopes.
- The structure and presentation of a TCRm epitope has important consequences related to target choice, mAb design, peptide and MHC restrictions, and possible cross-reactivity.

Figure 1. TCRm antibodies bind peptide/HLA class I complexes on the cancer cell

Proteins expressed in a cancer cell can be degraded by the proteasome and processed into peptides. Peptides are then loaded onto HLA class I molecules and are shuttled to the cell surface where they can be recognized by TCR on cytotoxic T cells. TCRm which mimic the specificity of TCR for peptide/HLA class I complexes can be designed or discovered to target these intracellular or “undruggable” proteins.

Table 1

Opportunities for TCRm antibodies

Target Class	Possible Proteins	Examples
Mutated oncogenes and tumor suppressors	Missense mutations in proteins Translocation-derived proteins	Ras, p53, Myc BCR-ABL
Aberrantly expressed self-proteins	Oncofetal antigens Cancer-testis antigens	WT1 PRAME, MAGE
Viral proteins	Retained intracellular viral proteins	CMV proteins, EBV proteins
Organ-specific secreted normal proteins	Secreted proteins	hCG, PSA
Lineage-specific normal proteins	Normal granule contents Lineage markers	Proteinase 3 Tyrosinase

Footnote

CMV: cytomegalovirus

EBV: Epstein-Barr virus

WT1: Wilms' tumor protein 1

PRAME: preferentially expressed antigen in melanoma

MAGE: melanoma-associated antigen

hCG: human chorionic gonadotropin

PSA: prostate-specific antigen

Table 2

Obstacles to overcome in TCRm antibody development

HLA restriction of epitope
Requirement for peptide processing and presentation
Low epitope numbers per cell
Cross-reactivities with similar linear sequence epitopes
Cross-reactivities with HLA molecules
Down-regulation of surface HLA molecules
Down-regulation of peptide presentation machinery
Alterations in proteasome function
Low target density hinders development of companion diagnostics

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 3

Selected difference between TCRm antibodies and other specific immunotherapies

Agent	Advantages	Disadvantages
TCRm	<ul style="list-style-type: none"> - Access to intracellular protein-derived epitopes - Long plasma T_{1/2} 	<ul style="list-style-type: none"> - Possible cross-reactive targets - Untested in human patients
mAb (traditional)	<ul style="list-style-type: none"> - Conformational epitope targets - Long plasma T_{1/2} - Well-characterized drugs with many examples in clinical use 	<ul style="list-style-type: none"> - Limited to extracellular targets
TCR	<ul style="list-style-type: none"> - Access to intracellular protein-derived epitopes 	<ul style="list-style-type: none"> - Low affinity - Short plasma T_{1/2} - Possible cross-reactive targets - Not drug-like
CAR T cell	<ul style="list-style-type: none"> - HLA-unrestricted - Access to nonprotein antigens 	Patient specific <ul style="list-style-type: none"> - Unpredictable pharmacokinetics - Uncertain or uncontrolled duration of effects
TCR engineered T cell or adoptive T cells	<ul style="list-style-type: none"> - Access to intracellular protein-derived epitopes 	<ul style="list-style-type: none"> - Patient-specific - Unpredictable pharmacokinetics - Possible cross-reactive targets - Uncontrolled duration of effects - Avidity and activity change with multiple surface contacts - May lead to autoimmunity
Cancer vaccine	<ul style="list-style-type: none"> - Active-specific therapy - Generally few adverse effects - May be long lasting 	<ul style="list-style-type: none"> - Less immediately potent - Duration of action is unpredictable and cannot be terminated easily