T cell receptor mimic antibodies for cancer therapy

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The major hurdle to the creation of cancer-specific monoclonal antibodies (mAb) exhibiting limited cross-reactivity with healthy human cells is the paucity of known tumor-specific or mutated protein epitopes expressed on the cancer cell surface. Mutated and overexpressed oncoproteins are typically cytoplasmic or nuclear. Cells can present peptides from these distinguishing proteins on their cell surface in the context of human leukocyte antigen (HLA). T cell receptor mimic (TCRm) mAb can be discovered that react specifically to these complexes, allowing for selective targeting of cancer cells. The state-of-the-art for TCRm and the challenges and opportunities are discussed. Several such TCRm are moving toward clinical trials now.

Cancer Antigens and T Cell Receptor-Mimic Monoclonal Antibodies

Spontaneous antitumor T cell responses occur frequently in cancer patients and analyses of these responses have led to successful identification of tumor antigens recognized by T cells in the context of HLA. Tumor specific antigens can originate from mutated gene products, such as Ras and BCR/ABL fusion proteins. Although the neoantigens resulting from these mutations are strictly unique to tumor cells, the chances that a peptide displaying such a mutation will bind the patient’s HLA and be displayed are small, and few are documented. Tumor associated antigens, on the other hand, include proteins that are overexpressed in tumor cells, and therefore are displayed at a far higher rate on the surface of cancer cells (Table 1). CD8+ T cells of the immune system can identify antigenic peptides presented by HLA class I molecules. Peptides recognized as non-self, such as those derived from mutated, oncofetal or viral genes, can be detected by T cells, which will then kill the antigen presenting tumor cell.

Strategies for enhancing T cell responses to these antigens include vaccination of cancer patients using DNA, peptides, whole proteins derived from tumor antigens, and dendritic cells loaded with peptides or incorporated with mRNAs. Unfortunately, therapeutic results to date have not been robust. Current approaches to primarily enhance tumor-specific T cell immunity by vaccination appear inadequate to maintain an effective antitumor immune response, likely because it is difficult to vaccinate patients against self-antigens. As our understanding of the complex interaction between tumors and the immune system has improved, alternative approaches have been exploited to enhance the therapeutic efficacy of T cells. One approach is to adoptively transfer T cells that have been engineered to express high affinity T cell receptors (TCRs) specific for tumor antigens. Another approach is to engineer T cells with chimeric antigen receptors (CARs). These constructs link antigen-specific mAb with one or more intracellular T cell co-stimulatory molecules. Transducing such constructs into polyclonal T cells directs T cell cytotoxicity to tumor cells. However, CAR T cells have been largely generated to recognized differentiation antigens that are already well recognized by mAbs. The CAR T cell may offer far more effective T cell therapy by bypassing immune tolerance to a predetermined antigen.

The other arm of adaptive immunity is circulating immunoglobulins, which have been effectively exploited therapeutically as mAbs. MAbs mediate their activity by direct cytotoxicity by blocking or activating signaling pathways, complement-dependent cytolyis, antibody-dependent cell cytotoxicity (ADCC), or by activating the immune response. The FDA has approved nearly 20 mAbs for the treatment of various hematological and solid tumors. Targets include primarily lineage and differentiation antigens such as epidermal growth factor receptor, vascular endothelia growth factor, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), PD1, CD20, CD30, and CD52. Immunoglobulins can also serve as carrier vehicles for targeted delivery of more potent cytotoxic agents, such as toxins, drugs, and radiation. However, all the marketed therapeutic mAbs have been limited to cell surface or extracellular proteins found on healthy cells and tissues, resulting in off-target toxicity. Additionally, as the vast majority of tumor specific and tumor associated antigens are intracellular, these important antigens cannot be targeted by conventional mAb therapy.

A TCRm mAb would be able to combine recognition of intracellular proteins, analogous to that of a TCR, with the therapeutic potency and versatility of a mAb. Biological and technical issues were major obstacles to this approach until
recently. The antigenic density of a peptide within a HLA class I molecule on the cancer cell surface (perhaps 10 to a few thousand molecules) is substantially lower than for most expressed cell surface targets (ranging from tens of thousands to a million molecules). In addition, the presented peptide is buried in the groove of the HLA molecule, a protein found in large numbers on the surface of all nucleated cells. Therefore, it was extremely difficult to generate a mAb with both high specificity and high affinity by traditional hybridoma techniques. Phage display library technology to select such mAbs has now made it possible to easily identify rare mAbs against epitopes such as peptide/HLA-A complexes, from a large library. As an example, we have successfully generated a fully human TCRm mAb, ESK1, specific for epitopes derived from WT1, an intracellular oncofetal antigen and transcription factor overexpressed in a wide range of cancer types. The mAb showed potent therapeutic activity against WT1-expressing leukemia and solid tumors, both in vitro and in vivo, via antibody-dependent cellular cytotoxicity.

**TCRm mAbs Under Investigation**

Numerous TCRm based constructs have been created over the past 15 years, recognizing both viral and self-antigens in the context of HLA-A, most commonly in HLA-A*02:01, and HLA-A*24:02. The majority of TCRm are used as biochemical tools to study antigen presentation, structure, and recognition. Many of these TCRm mAbs target viral antigen presentation, such as Env183/A2 (Hep B/HLA-A*02:01), KP14/1 and KP15/11 (HIV envelope gp160/HLA-A*02:01), and RL36A (West Nile Virus/mouse H-2D b). TCRm derivatives have also been designed for cancer therapy. In addition, TCR-like and TCR transduced T cells aimed at cancer antigens overexpressed in tumors, such as MAGE-A1 or WT1 respectively, have been described for engineered T cell therapy. TCRm Fab linked immunotoxin conjugates have been constructed as well. However, none of these TCRm have yet advanced to the clinical setting.

The generation of full-length functional TCRm IgGs for the purpose of cancer therapy has rarely been accomplished. Three groups have reported on such TCRm. The Weidanz lab has created three antibodies: RL6A (p68 RNA helicase/HLA-A*02:01), RL4B (hCG-β/HLA-A*02:01), and RL1B (Her2-E75/HLA-A*02:01). The Molldrem group created the TCRm mAb 8F4, targeting PR-1 in context of HLA-A*02:01. Finally,
our lab has generated the antibody ESK1, and its Fc enhanced form, ESKM, directed to WT1/HLA-A*02:01 (Fig. 1). The latter TCRm is a human IgG, allowing its use immediately in patients. While all of these TCRm antibodies have shown promising results in vitro and in vivo, clinical trials in humans are still pending.

Regulation of TCRm Epitope Expression

A potential hurdle to effective use of TCRm mAb is the relative lack of epitope density on the cell surface, which may be extremely low (100–1,000 sites per cell), as compared to the high (20,000–500,000 sites per cell) number of sites per cell for traditional mAbs. TCRm Abs have been shown to trigger complement mediated killing or ADCC even at low epitope density. Potency appears to be correlated with numbers of target sites, stressing the importance of examining epitope regulation.

Level of protein expression, protein half-life, peptide processing, HLA levels, and HLA presentation of the peptide all dictate TCRm target epitope density. The protein must first be translated in sufficient quantities to facilitate peptide processing, and stability plays an important role. For instance, defective ribosomal products (DRiPs) may accumulate due to errors in transcription, translation, or protein folding. Various reports suggest DRiPs are rapidly degraded and constitute a significant percentage of peptides presented on HLA. Short lived proteins also appear to be more likely to be present than those with a longer half-life. To initiate antigen processing, proteins are cleaved into random-sized peptides by proteasome-mediated degradation in the cytosol. These peptides then enter the endoplasmic reticulum through TAP, where they are trimmed by aminopeptidases, loaded onto HLA, and transported to the cell surface. Peptide HLA loading is governed by the binding affinity of the HLA protein to the peptide. The amino acid residues at position number 2 and 9 in the peptide determine binding affinity to most HLA alleles. Although several peptides may briefly bind a single HLA molecule, chaperones such as tapasin allow rapid peptide exchange ensuring the replacement of low-affinity peptides by those with high affinity. The resulting product is trafficked to the cell surface. Unstable peptides dissociate from HLA on the cell surface, resulting in significantly shortened half-lives. Moreover, specific peptides have been shown to cluster on the cell surface, which increases the avidity of the epitope and allows for increased activation of recruited effectors. However, some tumors have been observed to significantly downregulate surface HLA expression. Such tumors will be far less susceptible to TCRm based therapy due to target downregulation.

To overcome the issue of low target density, pharmacological modulation of key steps in peptide presentation should be considered. For instance, specific cytokines, such as IFNγ and TNFα, increase HLA presentation. Specific chemotherapeutic agents and radiation can also enhance HLA expression. Thus, modulation through exogenous treatment or cytokine release in the local tumor environment may increase epitope density and augment TCRm efficacy. Moreover, because tools for predicting affinity and immunogenicity of potential peptide epitopes are imprecise, development of robust tools will allow for greater accuracy in deciding which peptides will be presented in adequate numbers and hence lead to potential targets to pursue with TCRm Abs.

Determining Ideal TCRm Targets

The power of TCRm comes from their ability to exquisitely target cells based on expression of specific, otherwise undruggable, intracellular proteins. Ideal TCRm targets for cancer therapy are thus tumor-specific peptide/HLA complexes found in abundance on the cell surface. These antigens are most likely to be found in overexpressed proteins with short half-lives that are cleaved and processed into peptides with high affinity to the patient’s HLA.

Targets meeting the above criteria can be grouped into three general categories. The first category is antigens presented only by the tumor itself, such as peptides derived from commonly mutated proteins (e.g. KRAS G12V/D) or fusion proteins. Another group is re-expressed oncofetal proteins (e.g. Carcinoembryonic antigen) and overexpressed genes (e.g. MART-1/Melan-A, MAGE, PSA) (Table 1). Targeting commonly mutated proteins is the most conceptually interesting as it may enable the holy grail of cancer therapy: a drug which selectively kills transformed cells across a wide array of cancer types. The disadvantage of these common mutations is that not all tumors will bear susceptible mutations—or the correct HLA type. Developing TCRm against re-expressed oncofetal proteins (as we have done with WT1) or proteins overexpressed in cancer may be a more general strategy, in which the limited expression on healthy tissue must be addressed in each case.

A third broad category of possible TCRm targets are tumor associated cells of the microenvironment, such as regulatory T cells, tumor-associated macrophages (TAMs), and cells involved in angiogenesis. It is too early to tell whether targeting these tumor infiltrates will be an effective antitumor strategy, but it is notable that a phase I clinical trial targeting macrophages systematically with an anti-CSF-1R antibody has shown early positive results. As genes that distinguish tumor-infiltrating cells from their systemic counterparts are discovered—TCRm, which do not rely on surface marker expression—may allow for more specific targeting of only the tumor infiltrating cells. TCRm developed against populations of normal cells may have other applications, such as in diseases of immune dysregulation.

TCRm Versus TCR

While TCRm antibodies react with a peptide sequence similar to that which the TCR reacts with, (because both see an epitope within the peptide carried by HLA, as well as the HLA molecule...
Table 2. A Comparison of TCR and TCRm

| Feature                    | TCR                                | TCRm                                |
|----------------------------|------------------------------------|-------------------------------------|
| Binding domain             | Vα and Vβ                          | VH and VL                           |
| Affinity/avidity           | Typically > 1 μM; higher affinities may be engineered | 0.2–200 nM                         |
| Valency                    | Monovalent, unless on a cell       | Bivalent or monovalent              |
| Pharmacokinetics           | Variable; short                    | Days to weeks                       |
|                            |                                    | (for intact human IgG)              |
| Production                 | Ex vivo process, by vaccination, adoptive T cell therapy, or genetically modified T cell. | Large-scale manufacturing, well established. |
| HLA Subtype binding        | Highly restricted to patient’s HLA subtypes | Less stringent HLA restriction possible. |

Adoptive T cell and CAR therapies are far more expensive, cumbersome, logistically difficult to provide as off the shelf therapies. Furthermore, persistence of T cells is difficult to maintain in vivo.20,67 TCRm mAb are simple and inexpensive to manufacture, as has been done successfully for the past two decades for many FDA approved therapeutic antibodies. Additionally, TCRm mediated ADCC, complement killing, or TCRm immunocytokines rely on multiple effectors or no cellular effectors, respectively, and allows for the bypassing of T cell mediated immunosuppression in the cancer patients on chemotherapy.68

**Mechanisms of TCRm Function**

TCRm mAbs have been shown to mediate Fc-independent cell death, specifically such as: RL4B, RL6A, and RL1B.33,35 These TCRm antibodies activate apoptosis in target breast cancer models in which the hCG-β, p68 RNA helicase, or Her2 derived peptides are presented on HLA-A0201, respectively. This apoptosis is mediated by internalization of the antibody bound to the peptide/HLA complexes, followed by phosphorylation of the JNK protein kinase and/or p38 MAPK resulting in activation of the intrinsic caspase-dependent pathway. However, other therapeutic TCRm antibodies do not promote Fc-independent cell death.7,24

Fc-dependent mechanisms of mAb action can be further broken down into two subcategories: complement mediated or effector cell mediated. Complement proteins can bind to the Fc region of therapeutic mAbs and initiate the complement cascade, resulting in CDC. RL4B, RL6A, and 8F4 TCRm antibodies have been shown to induce complement in vitro.17,32,33,69 TCRm Abs also recruit immune effector cells, such as NK cells, monocytes/macrophages, and neutrophils, via Fcy receptors, resulting in ADCC. The exact mechanism of ADCC varies depending on the type of immune effector cell activated. For example, activated NK cells secrete perforin and granzyme B, which are taken up by the target cell and result in cell lysis, similar to cytotoxic CD8+ T cells.70 Meanwhile, monocytes and macrophages have been shown to phagocytose an IgG-bound target cell as the primary method of ADCC, but can also secrete cytotoxic factors, such as TNF and reactive oxygen intermediates.71,72 Neutrophils have also been suggested to mediate...
ADCC in this manner. ADCC has shown itself to be a widespread mechanism of action for therapeutic TCRm antibodies. RL4A, RL6A, and 8F4 have all demonstrated in vitro ADCC activity. Additionally, ESK, which does not show direct apoptotic induction nor complement activation, is a potent initiator of ADCC.

Challenges and Opportunities with TCRm

A potential concern for the efficacy of TCRm antibodies is the generally low density of their target peptide/MHC epitopes, with only hundreds to a few thousand expressed on a target cell surface, versus the tens to hundreds of thousands of epitopes targeted by commercially available antibodies. Therefore, strategies to augment the therapeutic index should be considered. For example, Fc engineering to enhance ADCC via altered Fc glycosylation or amino acid changes in the Fc sequence have been employed. In addition, TCRm mAbs serve as an ideal cancer targeting platform for delivery of cytotoxic payloads specifically to a tumor, including attachment of α emitting radioisotopes or potent drugs and toxins. Lastly, the ScFv’s used to reverse engineer a TCRm mAb can be formed into bispecific antibodies, bispecific T-cell engagers (BiTEs), and CARs for expression on cyotoxic T-cells.

The effective use of TCRm mAbs for treatment of cancer must overcome a number of hurdles, including the problems with low epitope density discussed earlier (Table 3). In addition, the determination of appropriate antigenic targets, the complete avoidance of cross-reactivity with the HLA molecule itself (found on all nucleated cells), the discovery of high specificity, high-affinity TCRm, and the resistance by tumors to ADCC are potential issues. In general, MHC molecules do not readily or rapidly internalize, reducing the efficacy of drug conjugates, so activation of immune effectors will likely be the dominant mode of cytotoxicity for TCRm. However, the low density of cell surface targets makes both ADCC and complement mediated killing far more difficult. Furthermore, some of the patients in whom the TCRm may be prescribed will not have sufficient numbers of active effector cells as a consequence of the underlying disease (such as with leukemias) or due to prior cytotoxic therapy.

Although the manufacture of mAb on a large scale is easily done, the selection of targets, mAb creation and verification are at this time all difficult, expensive, and time consuming. The selection of validated targets poses several challenges. HLA restriction has been posed as a major obstacle to the widespread use of TCRm. However, HLA-A*02 is found in up to 40% of Caucasians, and 10–20% of other ethnic groups around the world. The success of just one TCRm to this HLA type would treat more patients than a typical “targeted” therapy that can only reach the small fraction of patients that express a particular mutated kinase or receptor. By preparing several TCRm of different HLA restrictions, it would be possible to address a large fraction of patients of patients with many cancers.

While a number of transcription factors and proteins have been described as over-expressed in tumor cells, few are seen on the cell surface in the context of HLA in sufficient numbers to allow for TCRm guided ADCC. An approach to overcome this problem might be to utilize multiple TCRm to different epitopes presented by the same HLA type. Making multiple mAbs targeting a variety of commonly overexpressed targets in the context of a variety of the most common HLA types also would allow for increased specificity, efficacy, and population coverage. Another potential hurdle is the cross-reactivity of TCRm to epitopes that share sequence homology to the chosen 9-mer amino acid sequences. As the mAb binds to small numbers of amino acids derived from sequences from both the HLA and the peptide, other amino acid sequences (either from processed peptides that share partial homology, or from HLA that share homology) may bind the TCRm.

Conclusions

Effective, truly cancer-specific therapies do not currently exist. A major advantage of the TCRm approach is that for the first time it should allow a readily available and potent drug that binds almost exclusively to target cancer cells, a goal currently not

| Challenges for TCRm | Discussion and Possible Solutions |
|---------------------|----------------------------------|
| Low epitope density or low HLA | • Upregulate HLA or antigen pharmacologically, eg with interferon γ |
| Cross reactivity with HLA | • Increase potency of TCRm via Fc engineering, CAR, BiTE, etc. |
| HLA restriction | • Create predictive models in silico |
| Lack of MHC internalization | • Screen extensively during discovery |
| Reduced Effector cell numbers | • Identify the key contact residues of HLA molecules by crystallography |
| Cross reactivity with other epitopes | • Use of multiple TCRm to broaden coverage |
| | • Screen for HLA cross-reactive mAb |
| | • Use potent radiopharmaceutical that does not require entry such as α emitter |
| | • Careful choice of patient population, such as with reduced tumor burden |
| | • Use immunomodulators or radio-immunomodulators, CAR, or BiTE |
| | • Infuse human effector cells |
| | • Treat with GM-CSF, IL-2, or other cytokines to promote effector expansion |
| | • Careful screening and selection |
| | • Develop predictive models in silico |
| | • MASS Spec to confirm if the predicted epitopes are presented |
possible with any other approved form of therapy. Currently available small molecule therapies are not specific to cancer cells and other immunological approaches, such as vaccines or cellular therapies, which might be characterized as tumor specific, are cumbersome to produce, use, or control, or they lack the potency of the mAb. Moreover, as antibody therapy does not require the host adaptive immunity system, these drugs may be used in conjunction with lymphotropic chemotherapy. TCIRm may be viable and frozen or lyophilized, allowing widespread, immediate and inexpensive use worldwide in virtually any setting. Such accessibility is not possible with adoptive cell therapies, CAR T therapies, bone marrow transplants, or donor leukocyte infusions. However, as has been observed with traditional mAb therapy against widespread targets on cancer cells, mAb therapy alone is not likely to be sufficiently potent to eliminate 100% of cells when used as monotherapy, even at high effector to target ratios. Mechanisms of cancer cell resistance to antibody therapy must therefore be elucidated and remediated before the full potential of TCRm mAb therapy can be realized. Combination therapies with other anticancer agents likely will be necessary.

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No potential conflicts of interest were disclosed.

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