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TCR affinity for p/MHC formed by tumor antigens that are self-proteins: impact on efficacy and toxicity

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Abstract

Recent studies have shown that the range of affinities of T cell receptors (TCRs) against non-mutated cancer peptide/class I complexes are lower than TCR affinities for foreign antigens. Raising the affinity of TCRs for optimal activity of CD8 T cells, and for recruitment of CD4 T cell activity against a class I antigen, provides opportunities for more robust adoptive T cell therapies. However, TCRs with enhanced affinities also risk increased reactivity with structurally related self-peptides, and off-target toxicities. Careful selection of tumor peptide antigens, *in silico* proteome screens, and *in vitro* peptide specificity assays will be important in the development of the most effective, safe TCR-based adoptive therapies.

Introduction

Most of the reported tumor-associated epitopes for CD8+ T cells represent self-peptides presented by class I products [1]. While an immune response to upregulated self-antigens can be generated, the process of central tolerance in the thymus has evolved to eliminate T cells that express TCRs that react too strongly with these pepMHC [2,3]. The absence of peripheral T cells that might have reacted with a specific antigen has been referred to as the "hole in the repertoire". Recent studies with viral antigens, including HIV, raised the question of whether the collection of self-peptides that have structural homology to viral peptides might operate during negative selection to diminish the response to such foreign antigens [4,5]. When the target antigen is a self-peptide, a "hole in the repertoire" is even more likely. The ability to manipulate the affinities of TCRs, and to introduce TCRs into T cells for adoptive T cell therapies provides opportunities to overcome these limitations associated with negative selection against potential self-cancer antigens. Here we discuss the issues associated with raising the affinities of TCRs in order to drive robust T cell activity, and the potential risks this involves with TCR cross-reactivities and toxicities.

TCR affinities for self-protein epitopes

Although some peripheral T cells against tumor-associated self-peptides escape negative selection, it has been shown that their TCRs exhibit lower average affinities than typical

foreign antigens (mean K_d values of about 100 μM, compared to mean K_d values of about 10 μM for viral antigens)(Fig. 1) [6]. Nevertheless, naturally occurring TCRs that bind to tumor-associated pepMHC can mediate some clinical responses when introduced in a gene-modified, adoptive transfer setting [7]. TCRs with even modestly higher affinity can yield significant increases in potency [8], as it is believed that the affinities of wild-type TCRs for self-pepMHC tumor-associated epitopes may be too low for optimal $CD8^+$ T cell activity. Importantly, these wild-type TCRs do not mediate CD4 T cell activity [9–11], due to their absolute requirement for CD8 [9–15] (Fig. 1).

The importance of CD4 T cell activity in anti-tumor immunity has been established, but the ability to use transduced class I-restricted TCRs in establishing long-term CD4 T cell immunity remains to be shown. Nevertheless, re-direction of $CD4^+$ T cell effector activities against class I-restricted epitopes has been shown [12,14–16]. Higher affinity TCRs (e.g. K_D values of 1 μM or less) are required to mediate activation in $CD4^+$ T cells as compared to $CD8^+$ T cells, where CD8 can co-engage the class I MHC molecule. Effector $CD4^+$ T cells with higher affinity TCRs have mediated significant tumor control, and in some cases rejections, in mouse models [11,17–19]. TCRs with increased affinity against MART1/HLA-A2 [8], NY-ESO-1/HLA-A2 [20], MAGE-A3/HLA-A2 [21], and MAGE-A3/HLA-A1 [22], and ability to mediate in vitro $CD4^+$ T cell activity, have been evaluated in adoptive T cell transfer studies. While good persistence of $TCR^{+}CD4+$ T cells has been reported in some patients [8,20], the *in vivo* activity of the $CD4^+$ T cells bearing these receptors has not been fully characterized; thus, the extent of anti-tumor response, or even reactivity against normal tissues, that are mediated by $CD4^+$ compared to $CD8^+$ T cells is not clear. In summary, it remains to be determined if $CD4^+$ T cells with a class I-restricted T cells will persist, and/or be capable of recruiting the activity of $CD8^+$ T cells against other potential tumor antigens.

Despite their low affinity, wild-type TCRs can serve as leads to engineer mutations that confer higher affinity. For modest increases in affinity (probably only a few fold), individual site-directed mutations can be screened for T cell activity [14] or TCRs can be derived from allogeneic or xenogenic T cell clones [21,23,24]. For more significant increases in affinity, directed evolution strategies including yeast [25,26] or phage display [27] have been employed. As the TCRs generated by any of these strategies have not been through negative selection, appropriate screening strategies are necessary to assess specificity. This is especially important since CD8 contributes significantly to the ability of T cells to recognize class I complexes, even if the affinity of the TCR for a structurally similar self-peptide is as low as 300 μM [6,28].

The conserved docking orientation of TCRs on the pepMHC ligand [29,30] (Fig. 2) suggests that peptide specificity might best be retained by mutating CDR3 loops, which typically dock over the peptide. In contrast, CDR2 loops dock over the MHC helices and it is possible that mutations in these residues could promote non-peptide associated increases in binding affinity, and thus reduced peptide specificity. While these concepts are attractive in principle, in practice the binding of a TCR is complex, and the different CDR loops often act cooperatively. For example, CDR3 residues can engage MHC, either directly or through their action on other CDR loops. Furthermore, CDR1α often contacts the N-termini of peptides [31–33]), and thus it could be argued the CDR1α residues have the same potential

to confer peptide specificity as CDR3 loops. While there are no strict rules for engineering, avoiding affinity-enhancing modifications of CDR2 loops (Fig. 2) may be wise (e.g. although anecdotal, two patients treated with T cells expressing a CDR2-modified, MAGE-A3/HLA-A1-targeted TCR exhibited lethal effects apparently due to off-tumor off-target cross-reaction with a structurally-related titin peptide [22]).

Even when affinity mutations are limited to TCR regions that are predicted to contact the peptide, off-target cross-reactivity was observed in the first high-affinity TCR engineered in a mouse system [16], and in a more recent human TCR against the MAGE-A3/HLA-A2 complex, which cross-reacted with a peptide from MAGE-A12 [34]. To reduce affinities of engineered TCRs to an optimal level for recruitment of both CD8 and CD4 T cell activity, but minimizing the risk of peptide cross-reactivity, we have shown that key CDR2 residues that make energetically important contacts with MHC residues [35] can be mutated to easily generate a panel of TCRs with a range of reduced affinities [36,37].

Thresholds for efficacy and toxicity for on-target reactions

T cells with wild-type affinity TCRs can be extremely sensitive to foreign pepMHC, requiring only a few pepMHC complexes to initiate T cell triggering [38–40]. As indicated earlier, this sensitivity for a class I antigen is in part due to TCR synergy with the co-receptor CD8. The optimal TCR affinity range for safety and efficacy in adoptive therapies appears to depend to some extent on both the TCR and the target, but there are some general rules to follow based on a limited set of mouse and human studies.

Mouse studies

To study aspects of HLA-A2-restricted, tumor-associated epitopes in vivo, mice that express transgenic chimeric human-mouse class I MHC molecules, either A2-K^b [41] or AAD [42] have been used. Chimeric A2-K^b and AAD molecules both consist of human HLA-A2 α1 and α2 domains fused to mouse class I α3 domains from H2-K^b or H2-D^b, respectively. The mouse α3 domain allows binding of mouse CD8 and thus the full recognition and activation properties of mouse $CD8^+$ T cells. The chimeric molecules have been shown to present human HLA-A2-restricted epitopes, to bind to mouse CD8, and to elicit CTL responses in transgenic mice, allowing in vivo studies of responses to the HLA-A2-restrcited peptides [41,42].

A panel of gp100/HLA-A2-specific TCRs from vaccinated melanoma patients, spanning a K_d range from 1–100 μM were transduced into $CD8^+$ T cells from HLA-A2-K^b (α3 domain) transgenic mice [41,43]. The A2-K^b strain allows mouse CD8 to engage the class I HLA-A2/K^b molecule. When these T cells were used to treat tumors in A2-K^b mice, both control of tumor and ocular autoimmunity was enhanced with increasing TCR affinity, plateauing at a K_d value of 10 μM [43]. Unfortunately, the same TCRs were not tested in CD4 T cells to determine if the same higher affinity TCRs would mediate improved activity without the ocular effects.

Tyrosinase-specific CTL lines [44,45] and TCR-transgenic mice [46,47] were generated to study efficacy and safety. The high-avidity TCR called FH [42] recognizes human and

mouse versions of a tyrosinase$_{368–377}$ (which vary by a single Y to F mutation at the N-terminus) in the context of the human/mouse chimeric AAD MHC [41,42]. While transfer of the tyrosinase-specific CTL lines delayed the outgrowth of tyrosinase^{+} AAD^{+} tumors [45], on-target/off-tumor vitiligo caused by CD8 T cells was observed, and this was accelerated by depletion of CD25^{+} CD4^{+} T cells [44,46]. While the precise affinity of the FH TCR is not known, like the gp100 system enhanced binding properties that conferred good activity were correlated with more widespread on-target/off-tumor responses. By contrast, two TCRs that bound with enhanced affinity and conferred high functional avidity to a WT1 peptide presented by D^{b} did not shown signs of toxicity *in vivo* [48], despite reports that there are low levels of WT1 protein expression in lung and kidney tissues [49]. Hence, determination of an affinity threshold for on-target/off-tumor response is influenced by the distribution and no doubt expression levels of the target peptide.

Human studies

TCRs with various affinities have been used in human adoptive T cell trials with variable efficacy and safety. Notably, T cells expressing the DMF4 (K_d 170 μM) and DMF5 (K_d 40 μM) TCRs against MART-1/HLA-A2 have been used clinically to treat melanoma patients [7,8,31]. The higher affinity DMF5 TCR showed more robust *in vitro* and clinical antitumor response, but coincident with DMF5's improved anti-tumor response were toxicities due to on-target/off-tumor activity against MART-1 expressed in the eye, ear and skin [8]. Another anti-cancer TCR targeting the cancer-testis antigen NY-ESO-1 used a TCR (1G4-α95:LY) that had a 50-fold higher affinity for its target (K_d=730 nM) than the DMF5 TCR [14]. To date, no on-target/off-tumor effects have been reported in this trial [20]. The NY-ESO-1 antigen has a very restricted expression profile and is thought to be largely absent in normal adult tissues outside of testis. Again, the potential for on-target/off-tumor responses will rely heavily on the selection of target epitope.

Cross-reactivities mediating off-target toxicities

Relationships of TCR affinity and peptide cross-reactivity

In addition to on-target/off-tumor toxicities, TCR candidates for adoptive T cell therapies can exhibit off-target responses as described for the MAGE-A3 systems [22,34]. Both on-target and off-target activities are dependent on the potency of the T cell response, with higher affinity class I-restricted TCRs having the potential to mediate optimal CD8 T cell activity and also CD4 T cell activity. Off-target responses are impacted directly by TCR affinity due to the low affinity threshold necessary to mediate CD8-dependent T cell activity [28]. For example, as affinity for the tumor peptide is increased, affinities for structurally related peptides can also increase [16]. If such structurally related peptides are presented by the class I product on normal tissue at sufficient levels, there is the risk of off-target toxicities.

The issue of peptide cross-reactions by normal TCRs has long been considered important to allow responses to the diverse repertoire of potential epitopes [50,51]. It has been suggested that this cross-reactivity is due in part to the conformational plasticity of the CDRs of a TCR, combined with the low affinities required for CD8 T cell activity. Increasing the

affinity of a TCR by mutation of the CDRs can actually reduce this conformational flexibility, and thus can reduce cross-reactivity with structurally unrelated peptides [52]. However, increasing the affinity of the TCR enhances the affinity for structurally related self-peptide variants, as first reported with a nanomolar affinity-matured mouse TCR 2C [16]. It is this feature that is associated with a corresponding increased risk of activation by structurally related self-peptides [53]. Such off-target reactivity with structurally related self-peptides has been seen with engineered human TCRs [15,21] and led to the serious adverse effects in clinical adoptive T cell transfers [22,34].

In summary, the low TCR affinity threshold of CD8-dependent T cell activity dictates that extensive pre-clinical peptide analyses be performed with TCR candidates. The analyses should include activity screening against a battery of peptides and tissues: cognate peptide variants, proteome searches for similar peptides (see below), and tissue-representative cell lines [9,34,54]. Eventually it may be possible to include all peptides that have been validated as members of the immunopeptideome for a class I allele [55].

Proteome searches

Strategies to predict and screen potential self-peptides are crucial in the testing of TCRs that have optimal potential in adoptive T cell therapies. To err on the side of identifying such cross-reactivity, it may be useful to examine a panel of related TCRs with a range of affinities, including those with affinities even higher than the proposed final TCR candidate. As described above, TCR affinity can be easily reduced by mutating conserved CDR2 residues for the eventual clinical candidates [36,37].

Retrospective *in silico* proteome scanning based on the known reactivity of the MAGE-A3/HLA-A1-specific TCR revealed a peptide from titin that was recognized *in vitro* and, likely, *in vivo* [22,54]. In a prospective effort, we performed an in-depth, *in silico* analysis of over fifty tumor-associated, HLA-A2-restricted, 9-mer peptides from the Cancer Immunity Peptides Database (http://cancerimmunity/org/peptide/). To illustrate the analysis, Table 1 presents features of 19 selected tumor-associated peptides that are identical in humans and mice and that were isolated originally as HLA-A2 restricted antigens. Since MHC binding has been shown to be a critical parameter in judging the fitness of a T cell target peptide [56], each peptide was evaluated for predicted MHC binding via five algorithms (Table 1). The results showed general agreement among the HLA-A2 binding algorithms.

Each tumor peptide was also examined for sequences in the human and mouse proteomes that showed structural similarities. The scan was guided by a set of heuristics allowing conservative substitutions at every peptide position, plus allowing preferred MHC anchor residues at positions 2 and 9. Using these criteria, the number of unique, identified human peptides that were also predicted to bind to HLA-A2 varied greatly among these tumor epitopes, from as few as 3 to over 800 (Table 1). The number of identical mouse peptides, in anticipation of testing in mouse models, ranged from 2 to 330 (34 to 82%, depending on the cancer self-peptide). This suggests that testing candidate TCRs in models such as the AAD mouse will be useful as one pre-clinical approach to reveal cross-reactivity [11,17,47], especially for targets that are, themselves, identical in the mouse proteome. Of course, the quantitative presence of these peptides as class I complexes will depend also on the

transcription and translation of the genes, and on the processing pathways involved in antigen presentation [55].

Conclusions

Engineering TCR affinities against class I cancer peptide antigens provides an opportunity to optimize CD8 T cell activity, but also to recruit CD4 T cells against the cancer. The class I-restricted CD4 T cells can potentially facilitate induction of endogenous T cell responses against other cancer antigens, including patient-specific, mutated peptide antigens [57,58]. However, enhanced affinity of a TCR is associated with a higher risk of off-target peptide reactivity. The uniqueness of a cancer peptide (i.e. fewer structurally related peptides) may be a useful parameter to consider with peptide targets, along with tissue-expression profiles, tumor-expression levels, and links to oncogenicity [1]. Pre-clinical screens for cross-reactivity should include the panel of *in silico* identified structurally related peptides. As other factors also determine processing and presentation of peptides by class I MHC products, it will eventually be useful to focus *in vitro* screens on peptides that have been validated as class I complexes, which would allow non-structurally related peptides to be included in a much broader screen [55,59].

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Highlights

- Engineered TCRs can overcome a “hole in the T cell repertoire”.
- Affinity tuning can yield optimal TCR candidates for adoptive T cell therapy.
- On-target/off-tumor toxicity can be revealed by more potent T cells.
- Higher affinity TCRs can result in off-target reactivity with related peptides.
- Pre-clinical screening methods for off-target T cell activity are critical.

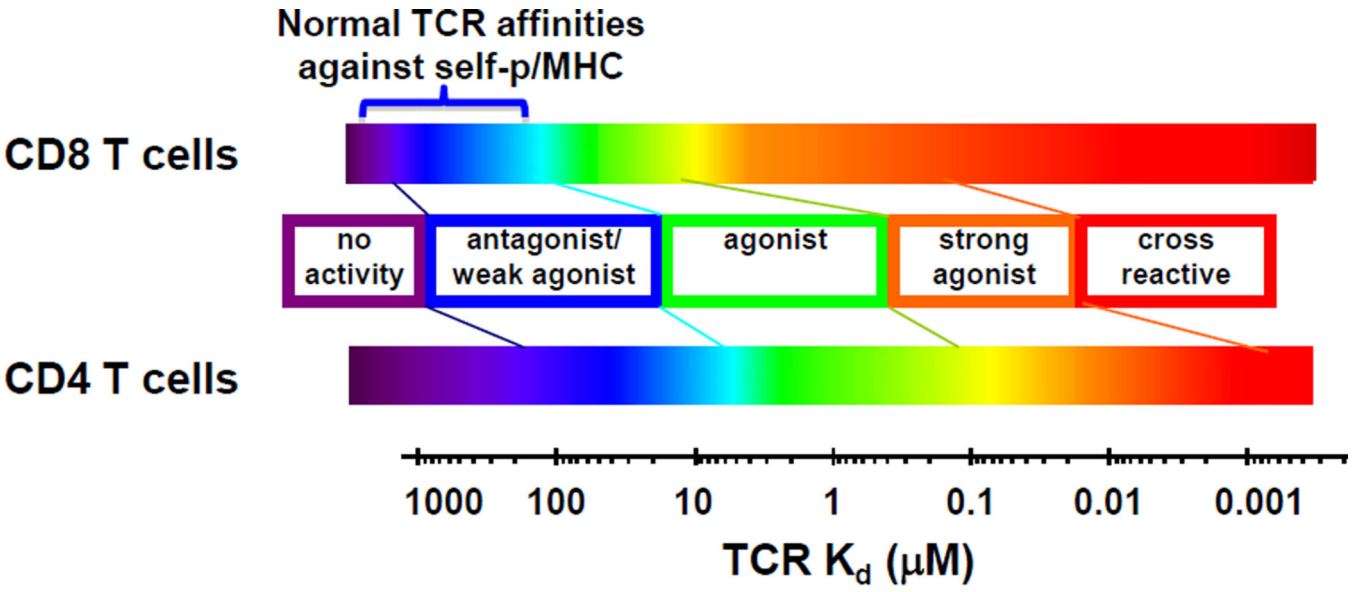

Figure 1. Relationship between TCR affinity and T cell activity for CD8 and CD4 T cells

TCR affinity for class I pep/MHC impacts T cell activity for CD8 (top) and CD4 (bottom) T cells. CD8 T cells are more sensitive at weaker affinities, due to the contribution of the CD8 co-receptor to class I MHC binding, regardless of bound peptide. At higher affinity ranges, peptides that are structurally similar to the cognate peptide (tumor antigen in the present review) are capable of functionally cross-reacting with the T cell.

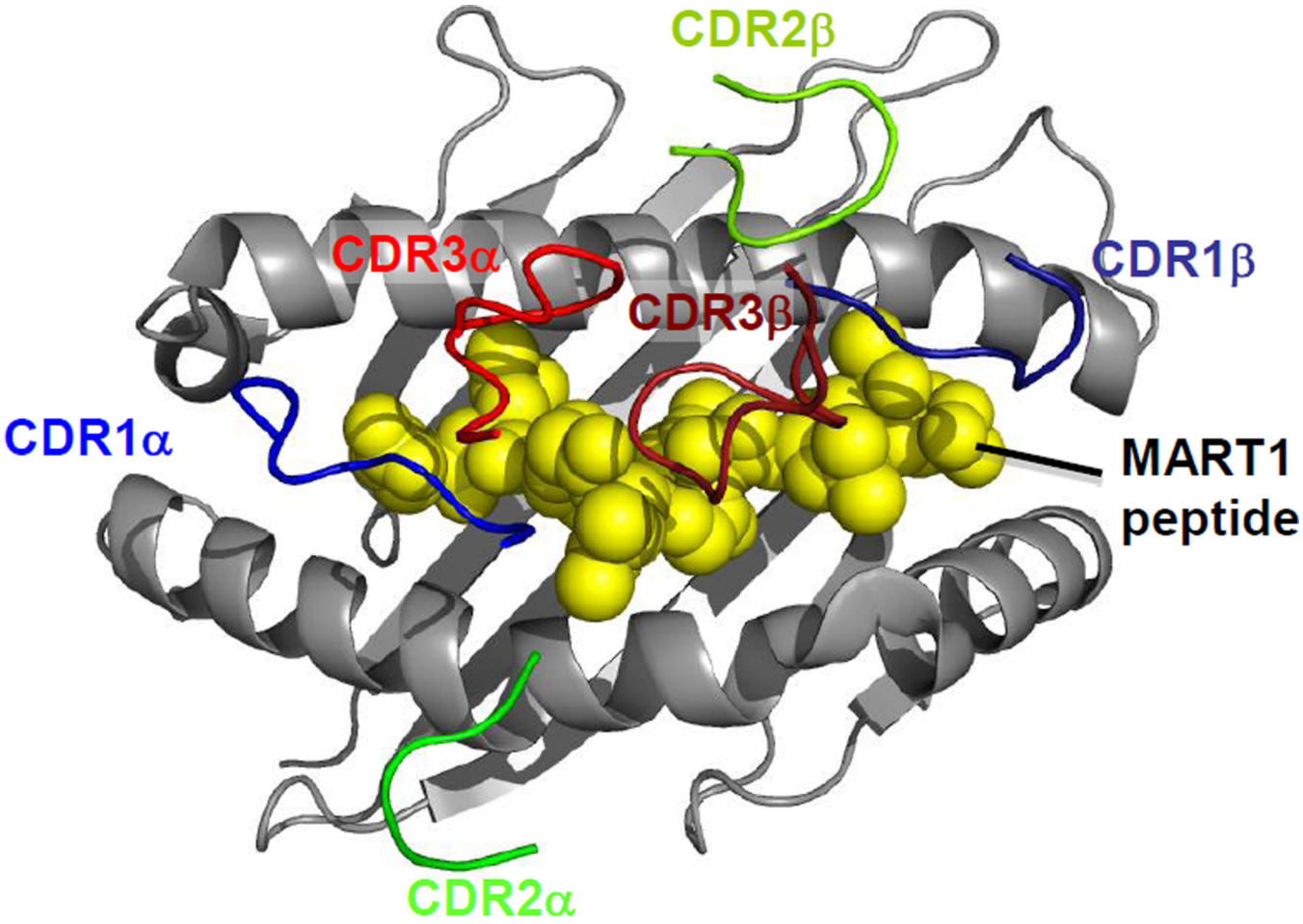

Figure 2. Conserved TCR docking onto pepMHC

CDR loops of the DMF5 TCR (PDB ID=3QDG) are shown binding to MART1/HLA-A2 as an example of the highly conserved docking geometry observed between TCRs and MHCs. HLA-A2 is shown in gray; MART1 peptide is shown in yellow. CDR3 loops (CDR3α in red, CDR3β in brick red) dock predominately over peptide, while CDR2 loops (CDR2α in green, CDR3β in lime green) predominately contact MHC. CDR1 loops (CDR1α in blue, CDR1β in navy) can contact both the MHC and the peptide; the CDR1α loop in particular frequently makes specific contacts with the N-terminus of the peptide.

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Table 1

Binding predictions and frequency of similar peptides for tumor-associated epitopes.[a]

Tumor Associated Protein	peptide sequence[b]	SYFPEITHI[c]	BIMAS[d] half-life (s)	ANN binding[e] IC50 (nM)	SMM binding[f] IC50 (nM)	NetMHCpan[g] (nM)	Compiled binding prediction score[h]	# unique binding peptide matches[i]	% peptide matches in mouse[j]
glypican-3	FVGEFFTDV	18	830	30	80	40	6.5	3	67
TRP-2	SVYDFFVWL	21	970	20	60	20	6.5	4	75
RAB38 / NY-MEL-1	VLHWDPETV	23	60	50	110	100	5	4	75
EZH2	FMVEDETVL	21	120	20	40	20	6	9	44
cyclin-A1	FLDRFLSCM	20	80	40	70	40	5.5	11	82
HER-2/neu	RLLQETELV	24	130	30	80	40	6	11	55
HER-2/neu	TLEEITGYL	23	4	220	430	230	2.5	12	42
KIF20A	CIAEQYHTV	25	60	100	200	80	5	30	57
WT1	CMTWNQMNL	17	20	650	480	400	2	35	34
HER-2/neu	HLYQGCQVV	23	3	110	130	100	4	41	34
HER-2/neu	KIFGSLAFL	28	480	10	60	20	6.5	42	38
gp100	ITDQVPFSV	18	4	70	150	200	3.5	48	44
WT1	RMFPNAPYL	22	320	7	30	10	6.5	50	56
ENAH (hMena)	TMNGSKSPV	21	50	280	290	430	3	55	47
Proteinase3 (PR1)	VLQELNVTV	28	490	10	30	10	7.5	82	44
CPSF	KVHPVIWSL	23	110	50	130	60	4.5	86	57
cyclin D1	LLGATCMFV	22	650	20	30	20	7	266	37
PAX5	TLPGYPPHV	24	70	20	40	10	6	341	37
PAP	TLMSAMTNL	21	180	10	60	10	6	818	40

[a] Shaded cells indicate the strength of HLA-A2 binding predicted for each algorithm: Strong binding = orange; Moderate binding = gold; weak binding = pale yellow; predicted not to bind = no shading.

[b] Selected 9-mer tumor-associated, HLA-A2-restricted peptides from the Cancer Immunity Peptide Database (http://cancerimmunity.org/peptide/).

[c] SYFPEITHI binding scores from online server (http://www.syfpeithi.de/).

[d] BIMAS binding predictions from BioInformatics and Molecular Analysis Section (NIH) online server (http://www-bimas.cit.nih.gov/molbio/hla_bind/).

[e] ANN binding predictions from the Internet Epitope Database (IEDB) Analysis Resource (http://tools.immuneepitope.org/mhci/).

[f] SMM binding predictions from the Internet Epitope Database (IEDB) Analysis Resource (http://tools.immuneepitope.org/mhci/).

[g] NetMHCpan binding predictions from NetMHCpan2.8 Server (www.cbs.dtu.dk/services/NetMHCpan/).

[h] Binding score derived from describing other predictions as not binding (0), weak binding (0.5), moderate binding (1), or strong binding (1.5). The sum of these descriptive scores from each prediction was calculated for each target.

[i] Search for all non-redundant structurally similar 9-mer peptides in the complete Homo sapeins proteome from The Universal Protein Knowledgebase (UniProtKB). Number indicates unique matches predicted to bind at least weakly to HLA-A2 by ANN and SMM.

[j] Percentage of peptides from the human proteome structurally similar to the target (above) which have identical sequences in the Mus musculus proteome.