# It's the Peptide-MHC Affinity, Stupid

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**Adoptively transferred T cells can reject large established tumors, but recurrence due to escape variants frequently occurs. In this issue of Cancer Cell, Engels et al. demonstrate that the affinity of the target peptide to the MHC molecule determines whether large tumors will relapse following adoptive T cell therapy.**

Tumor rejection usually requires $CD8^+$ effector T ($T_E$) cells, which recognize complexes of peptide bound to major histocompatibility complex (MHC) I molecules (pMHC) on tumor cells using their T cell receptor (TCR). The peptide is the proteasomal degradation product of the tumor antigen (TA). Upon recognition of pMHC, $T_E$ cells produce effector molecules such as perforin, IFN-γ, and TNF. Although it has long been believed that direct killing of cancer cells by $T_E$ cells alone is responsible for tumor rejection, recent studies show that destruction of the tumor stroma by $T_E$ cells is critical for tumor eradication, with different mechanisms being discussed (Anders and Blankenstein, 2013; Schreiber, 2013 and references therein). While adoptively transferred $T_E$ cells can reject large established tumors in experimental models and in humans, tumor recurrence after initial regression is a major obstacle. To overcome this problem, research has focused on improving T cell function by in vitro TCR affinity maturation, which can result in the loss of T cell fitness or specificity (Engels et al., 2012; Stone et al., 2009). Little attention has been paid to another variable in the three-molecule interaction: the affinity of the peptide to the MHC (pMHC affinity). This is surprising, given that the affinity of the TCR for pMHC usually lies within a narrow range (1–100 μM) due to the complex T cell selection process, while pMHC affinity ranges between <1 nM to >20,000 nM due to the partly stochastic nature of peptide sampling (Figure 1).

In this issue of *Cancer Cell*, Engels et al. (2013) addressed the impact of pMHC affinity on tumor eradication versus relapse. In a reductionist approach, they expressed the same amount of different peptide antigens in the same cancer cells. The peptide antigens were derived from mouse and human TA, presented by H-2 or HLA molecules, respectively, and all known as T cell epitopes. Mice with large tumors containing abundant stroma were then treated with TCR-transgenic $T_E$ cells specific for the respective pMHC complex so that the peptide was the only variable. Therapy outcome correlated perfectly with pMHC affinity (Engels et al., 2013; Figure 1). pMHC affinities <10 nM ($IC_{50}$ as measured by cell-free assays) elicited tumor rejection, and those with >100 nM caused relapse. Only high-affinity peptides were cross-presented by tumor stroma cells, causing stroma destruction and thereby preventing antigen loss variants. Peptides with very low affinity to MHC ($IC_{50}$ ~22,900 nM) could not even induce the selection of escape variants, and antigen-positive tumors progressed. In vitro T cell kill assays did not predict rejection epitopes. In the clinic, the reasons for tumor relapse often remain unknown. Based on this study, experiments in HLA-transgenic mice with established $HLA^+$ syngeneic mouse tumors expressing human TA may allow prediction of epitopes that should or should not be targeted clinically. These mice can be treated with mouse $T_E$ cells specific for the human TA to ask whether tumors will be rejected or recur. The human TA-specific mouse $T_E$ cells can be generated by the transfer of TCR genes, which can be isolated against virtually any human TA from the nontolerant repertoire, termed TCR gene therapy (Anders and Blankenstein, 2013; Schumacher, 2002).

Another important question addressed by Engels et al. (2013) is: how useful are many of the TA epitopes currently employed clinically as therapeutic targets? T cell epitopes of human TAs are usually defined by autologous systems. Most TAs are self-proteins (self-TA), and only low-avidity T cells that survived tolerance mechanisms are in the normal repertoire. Typically, one or few TA epitopes for a given MHC restriction molecule are described. Algorithms quite accurately predict pMHC affinity, and the $IC_{50}$ values determined by cell-free assays in Engels et al. (2013) did not differ greatly from the predicted values. Many of the described T cell epitopes of human self-TA have low affinity, e.g., $IC_{50}$ for NY-$ESO_{157–165}$ (HLA-A*02:01): 1,262 nM, Melan-A/MART-$1_{26–35}$ (HLA-A*02:01): 7,600 nM, or MAGE-$A1_{161–169}$ (HLA-A*01:01): 165 nM, as predicted by Immune Epitope Database Analysis Resource. Peptides predicted with high affinity have rarely been described as T cell epitopes. Although it is possible that some of these peptides are not generated (processed and presented), it is more likely that these T cells have been deleted during negative selection in the thymus, if one assumes that with increasing pMHC affinity the immunogenicity of the epitope and the risk of autoimmunity also increase. Thus, the best, but also the most dangerous, epitopes as targets for T cell therapy may not yet be known. Conversely, cancer vaccines targeting self-TA face the difficulty of not only relying on T cells, which survived central tolerance, but also targeting epitopes of low pMHC affinity.

A critical issue in this study is the source of the TCRs expressed by the transgenic $T_E$ cells and whether the TA is of self or non-self origin. The TCRs specific for high-affinity pMHC (SIY, ovalbumin, and

tyrosinase) were derived from an antigen-free (nontolerant) host, whereas the TCR specific for low affinity pMHC (gp100) was isolated from an antigen-positive (tolerant) host. Even if the human gp100 peptide (sharing six of nine amino acids with mouse gp100) was of non-self origin for the mouse T cells, it is unclear whether the TCR affinity for this pMHC is comparable to those TCRs from the nontolerant repertoire, recognizing pMHC with high affinity. Thus, one cannot exclude that a higher affinity of the TCR for pMHC, isolated from the non-tolerant repertoire and used for TCR gene therapy, can, at least partially, compensate for lower pMHC affinity.

The experiments targeting the self-TA gp100 with low pMHC affinity reflected a clinical TCR gene therapy trial with transient autoimmunity and little efficacy (Johnson et al., 2009), indicating that the experimental cancer model can predict clinical success/failure. The experiments targeting tyrosinase, another melanocyte differentiation antigen with high pMHC affinity, self for the host and non-self for the $T_E$ cells resulted in tumor rejection and autoimmune vitiligo. However, the severity of autoimmunity may be difficult to predict using the mouse model and depends greatly on the respective self-TA. As noted earlier, unforeseen expression of self-TA on rare vital cells is an unresolved problem when using TCRs from the nontolerant repertoire that target TA with assumed restricted tissue expression such as differentiation or cancer-testis antigens (Blankenstein et al., 2012). TCR from the nontolerant repertoire can be biological weapons (Bos et al., 2008). However, we hypothesize that the thymus overshoots in deleting more T cells than necessary. The repertoire is still large enough to cope with most pathogens, and the evolutionary priority was to minimize the risk of autoimmunity. There may be a useful compartment of T cells against self-TA in the nontolerant repertoire that cause little or no damage but are nevertheless deleted. Nonetheless, targeting somatically mutated non-self TA would clearly be advantageous (Anders and Blankenstein, 2013; Schreiber and Rowley, 2008).

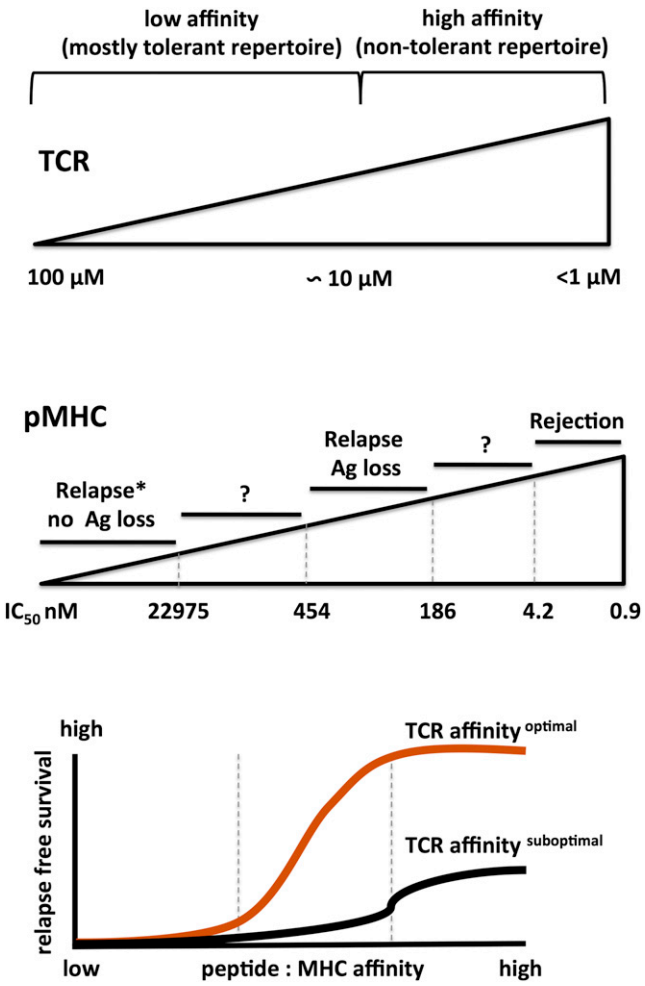


**Figure 1. Tripartite Molecular Interaction Influences whether T Cells Reject Large Established Tumors or Select Escape Variants**

Top: the range of affinities of the T cell receptor (TCR) to peptide/MHC I complexes (pMHC) is narrow (1–100 μM). TCRs specific for self-antigens (tolerant repertoire) tend to have lower affinity, whereas TCRs specific for non-self antigens (non-tolerant repertoire) tend to have a higher affinity for their cognate pMHC.

Middle: the range of pMHC affinities is broad (<1 nM to >20,000 nM). Indicated are pMHC affinities used in Engels et al. (2013) relative to therapeutic outcome. With decreasing pMHC affinity, adoptive T cell therapy leads from rejection to regression/recurrence of antigen (Ag) loss variants and then to recurrence without antigen loss (*or therapy failure). Note the gap in affinities between the different therapeutic outcomes, e.g., between 4.2 and 186 nM. For a variety of human tumor antigens, T cell epitopes are predicted within this range, raising the question of the cut-off value of "rejection epitopes".

Bottom: the relationship between pMHC affinity and success of T cell therapy is shown. If the TCR to pMHC affinity is optimal (red line), increasing pMHC affinity will (from a certain threshold onward) lead to an increase of success in therapy (relapse free survival) until a plateau (100% success) is attained. Black line, hypothetical graph illustrating that not only pMHC affinity but also TCR affinity decides over rejection versus relapse.

The study by Engels et al. (2013) is important, because it teaches us which epitopes not to target and how relevant experimental cancer models can be. However, a high affinity pMHC is not a good target per se. Too-low TA expression, inefficient processing and peptide presentation or posttranslational modification of the peptide could impede T cell therapy despite targeting a high-affinity pMHC. TAs are not always homogenously expressed within the tumor. In this case, the mechanism of tumor stroma destruction and the extent of bystander elimination of escape variants need to be better understood. Together, suitable TAs and particularly epitopes as targets in adoptive T cell therapy can and should be selected based on rational experimental models before clinical tests are done.

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## REFERENCES

Anders, K., and Blankenstein, T. (2013). Clin. Cancer Res. *19*, 320–326.

Blankenstein, T., Coulie, P.G., Gilboa, E., and Jaffee, E.M. (2012). Nat. Rev. Cancer *12*, 307–313.

Bos, R., van Duikeren, S., Morreau, H., Franken, K., Schumacher, T.N., Haanen, J.B., van der Burg, S.H., Melief, C.J., and Offringa, R. (2008). Cancer Res. *68*, 8446–8455.

Engels, B., Chervin, A.S., Sant, A.J., Kranz, D.M., and Schreiber, H. (2012). Mol. Ther. *20*, 652–660.

Engels, B., Engelhard, V.H., Sidney, J., Sette, A., Binder, D.C., Liu, R.B., Kranz, D.M., Meredith, S.C., Rowley, D.A., and Schreiber, H. (2013). Cancer Cell *23*, this issue, 516–526.

Johnson, L.A., Morgan, R.A., Dudley, M.E., Cassard, L., Yang, J.C., Hughes, M.S., Kammula, U.S., Royal, R.E., Sherry, R.M., Wunderlich, J.R., et al. (2009). Blood *114*, 535–546.

Schreiber, H. (2013). Cancer Immunology. In Fundamental Immunology, Seventh Edition, W.E. Paul, ed. (Philadelphia, PA, USA: Lippincott Williams and Wilkins, a Wolters Kluwer business), pp. 1200–1234.

Schreiber, H., and Rowley, D.A. (2008). Science *319*, 164–165.

Schumacher, T.N. (2002). Nat. Rev. Immunol. *2*, 512–519.

Stone, J.D., Chervin, A.S., and Kranz, D.M. (2009). Immunology *126*, 165–176.

# Standing Out from the Crowd: Cancer Stem Cells in Hepatocellular Carcinoma

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**Cancer stem cells (CSCs) drive solid tumor formation. In this issue of *Cancer Cell*, Zhao and colleagues identify the calcium channel α2δ1 subunit as a new functional hepatocellular carcinoma (HCC) CSC biomarker, which is vital for CSC biology as blocking α2δ1 in combination with doxorubicin treatment hinders HCC tumor formation.**

Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancers and is the third most common cause of cancer-related deaths worldwide (Edwards et al., 2010). Unlike most other carcinomas, where mutations in specific oncogenes or tumor suppressors drive tumor initiation and progression, the majority of HCCs are multifactorial and primarily due to infections with hepatitis B virus (HBV) or hepatitis C virus (HCV). However, worldwide cases of nonviral HCC are on the rise due to growing numbers of patients with metabolic liver diseases (Alberti et al., 2005; Van Thiel and Ramadori, 2011). This multi-causality makes identification and subsequent targeting of a common HCC-specific alteration or even a cell-of-origin virtually impossible. Fortunately, where consensus does exist is in the concept that the majority of HCC arise from a subpopulation of cancer cells referred to as tumor-initiating cells (TICs) or cancer stem cells (CSCs) (Majumdar et al., 2012). Thus, identifying and therapeutically targeting these cells represents a more feasible approach for treating HCC regardless of the underlying cause.

CSCs are believed to possess stem cell-like properties such as unlimited self-renewal, exclusive in vivo tumorigenicity, and subsequent generation of differentiated progeny recapitulating the parental tumor phenotype (Figure 1). Evidence for their existence in several solid tumors has been experimentally demonstrated (reviewed in Hermann et al., 2010). For HCC, cells expressing diverse markers such as CD133, CD13, CD24, CD90, and EpCAM as well as cells defined as the side population have all been demonstrated to bear CSC characteristics. Apparently, the utility of these different markers across established cell lines and primary tumors varies significantly, and their suitability for therapeutic targeting has not been extensively evaluated. Therefore, the identification of markers, preferably a single marker, for efficient isolation of CSCs from the complex tumor cellular environment across different HCC tissues is still critically needed.

In this issue of *Cancer Cell*, Zhao et al. (2013) report that HCC CSCs can be specifically isolated with a new antibody (1B50-1) identified using a whole-cell subtractive immunization approach that recognizes the isoform 5 of the cell surface calcium channel α2δ1 subunit. 1B50-1 binds a subpopulation of HCC cells, hereafter termed $\alpha2\delta1^+$ cells, exhibiting stem cell-like properties, such as increased invasiveness, expression of stem cell-associated genes (*OCT4*, *SOX2*, *NANOG*, and *BMI1*), increased self-renewal, and the ability to give rise to both $\alpha2\delta1^+$ and $\alpha2\delta1^-$ cells. More importantly, the authors showed that subcutaneously injected $\alpha2\delta1^+$ cells from cell lines and primary HCC tumors were more tumorigenic in NOD/SCID mice compared to their $\alpha2\delta1^-$ counterparts. Although the increased tumorigenic potential of $\alpha2\delta1^+$ cells was evident with as little as $10^3$ cells, limiting dilution assays (injection with less than 100 cells were not performed) revealed that not all $\alpha2\delta1^+$ cells were tumorigenic (TIC frequency in primary cases: 1 in 458 [748-281]), and higher numbers of $\alpha2\delta1^-$ cells were also capable of forming tumors (TIC frequency: 1 in 1,957 [3,785-1,012]) (calculated from Table 1 in Zhao et al., 2013). Therefore, $\alpha2\delta1^+$ cells from primary tumors were enriched for CSCs 4-fold.

Unlike many normal tissues where a stringent unidirectional hierarchy and strict balanced asymmetric division preserve tissue integrity (Jan and Jan, 1998), data in solid tumors are generally not as clear cut. On the one hand, this might be related to our still limited ability