Shared cancer neoantigens: Making private matters public
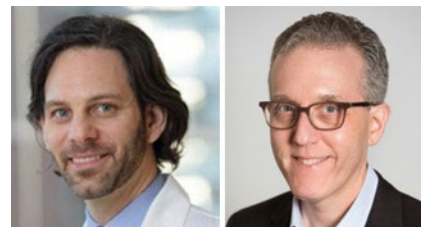
In this issue of JEM, Chheda et al. (https://doi.org/10.1084/jem.20171046) report that a conserved hotspot mutation associated with an aggressive form of brain cancer generates an immunogenic T cell epitope restricted by a common HLA subtype, thereby creating a "public" neoantigen.

T cells are the primary effectors responsible for causing tumor regression in the majority of successful cancer immunotherapies. Detailed immune monitoring studies have been performed on exceptional patient responders to either immune checkpoint inhibitors or adoptive cell transfer of tumor-infiltrating lymphocytes (Robbins et al., 2013; van Rooij et al., 2013; Snyder et al., 2014; Tran et al., 2014, 2016; Rizvi et al., 2015; Stevanović et al., 2017). These analyses revealed that T cell responses in these patients can target peptides derived from proteins resulting from nonsynonymous somatic mutations. Newly created antigens resulting from cancer-specific mutations, or neoantigens, pose an unprecedented challenge to developing antigen-specific immunotherapies. The human exome is ∼30 megabases in size. Consequently, the chance that any single random somatic mutation will recur in more than one patient is exceedingly small. This fact, combined with the requirement that a mutated protein can only be detected by T cells if it is processed in the proteasome and presented by one of the patient's complement of HLA molecules, effectively means that most neoantigens are patient specific. Immunotherapies that seek to raise an antigen-specific immune response to such "private" neoantigens must therefore be customized for each individual patient, creating substantial practical and regulatory hurdles (Klebanoff et al., 2016).

However, not all somatic mutations occur at random. Mutations that alter protein function to promote oncogenesis, so-called driver mutations, can systematically reappear across patients. Further, these function-altering mutations typically occur in tightly constrained hotspot regions within a protein. Moreover, only a single or limited number of amino acid residues can be substituted at these hotspots and still cause altered function. Finally, because driver mutations often arise early during cellular transformation and are required to maintain the malignant phenotype, they tend to be clonally conserved across metastases (Makohon-Moore et al., 2017). The integrated effect of these factors is that if a peptide containing a hotspot mutation is bound by a relatively common HLA allele, an ideal "public" neoantigen shared across patients would be created. Given the extraordinary precision with which a peptide binds the groove of an HLA molecule, it is not likely that a hotspot mutation–containing peptide will bind to any HLA allele, let alone a commonly represented one (Falk et al., 1991). Thus, discovering a "public" neoantigen is akin to winning the proverbial immunotherapy lottery: the odds are long but the potential rewards can be handsome. In the manuscript reported in this issue, the research team led by Hideho Okada demonstrates that they have purchased just such a "winning ticket" (see Chheda et al.).

It was recently discovered that most diffuse midline gliomas (DMGs), a universally fatal childhood brain cancer, harbor a hotspot mutation resulting in the substitution of a methionine for a lysine residue at position 27 of histone variant H3.3 (H3.3K27M; Mackay et al., 2017). This alteration disrupts the activity of the histone methyltransferase Polycomb Repressive Complex 2, leading to a global reduction in inhibitory H3K27me3 levels, aberrant gene expression, and ultimately, brain tumorigenesis. Based on these findings, Chheda et al. (2018) used an HLA binding prediction algorithm to assess whether a peptide containing the hotspot H3.3K27M mutation might bind to any MHC class I molecule.
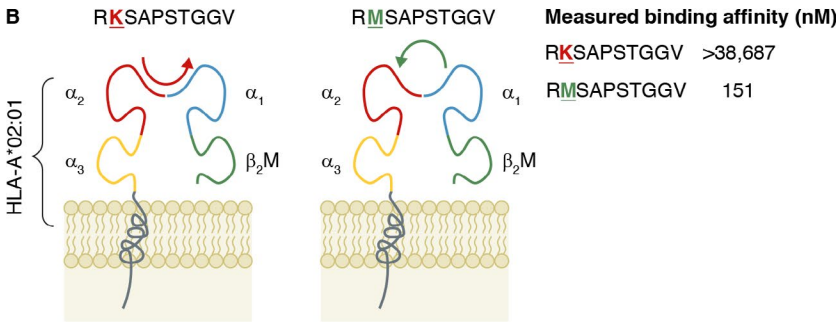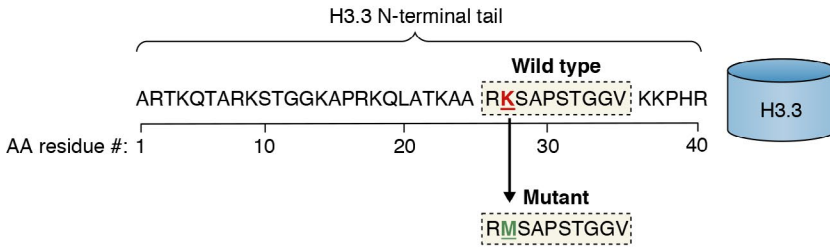
These analyses suggested that a 10-mer peptide encompassing residues 26–35 of H3.3K27M (H3.3K27M$_{26-35}$) would bind the prevalent MHC class I allele HLA-A*02:01 (HLA-A2) with nearly 1,000-fold higher affinity relative to the corresponding WT sequence. This in silico prediction was confirmed using a competitive peptide-binding assay, demonstrating that the mutant peptide can displace the WT peptide from a recombinant HLA-A2 molecule. Of note, the lysine to methionine substitution in the mutant peptide fortuitously occurred in the second position. Prior experiments demonstrated that aliphatic amino acid residues, such as methionine or leucine, can dramatically enhance peptide binding affinity to HLA-A2 when present in the second position without interfering with TCR contact sites (Falk et al., 1991). Therefore, the K to M substitution had the functional effect of unmasking an epitope to make it visible to T cells.

Chheda et al. (2018) next performed in vitro sensitization using the H3.3K27M$_{26-35}$ peptide pulsed onto peripheral blood leukocytes taken ei-

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A Creation of a public neoantigen resulting from the H3.3K27M hotspot mutation

B

C

	Cancer neoantigens	
	Private	**Public**
Frequency	Patient specific	Shared
Origin	Typically passenger mutations	Typically driver mutations
Potential for clonal heterogeneity	High	Reduced
Potential for selection pressure	High	Reduced
Mechanisms of antigen escape	Intratumoral heterogeneity, LOH for restricting HLA allele, antigen processing defect	LOH for restricting HLA allele, antigen processing defect
Examples	–	H3.3K27M / HLA-A*02:01 KRAS G12D / HLA-C*08:02 KRAS G12V / HLA-A*11:01 CDK4 R24C / HLA-A*02:01 CDK4 R24L / HLA-A*02:01

LOH, loss of heterozygosity

The mechanistic basis for the creation of a public neoantigen shared across patients and presented by a prevalent HLA molecule. (A) The majority of diffuse midline gliomas harbor a hotspot mutation resulting in substitution of a methionine for a lysine residue at position 27 of histone variant H3.3 (H3.3K27M). (B) A 10–amino acid peptide harboring this K to M substitution in the second position generates a heteroclitic epitope with a superior binding affinity to the MHC class I molecule HLA-A*02:01. This in turns permits efficient T cell recognition. (C) Table comparing the attributes of patient-specific private neoantigens and shared public neoantigens.

ther from HLA-A2[+] healthy donors or patients with DMG in an attempt to raise hotspot neoantigen–specific T cells. After T cell expansion, the team screened for reactivity against H3.3K27M$_{26-35}$ peptide or the corre-sponding WT sequence. In 3/3 brain cancer patients, they found evidence of specific cytokine release by T cells to the mutant but not the WT sequence. In contrast, none of the tested healthy donors exhibited a response to either H3.3-derived peptide, suggesting that the tumor-bearing state causes T cell priming and expansion. By staining pools of expanded T cells with an HLA-A2 tetramer loaded with the H3.3K27M$_{26-35}$ peptide, the authors then isolated antigen-specific T cells by FACS sorting. Subsequent expansion of isolated T cells afforded one clone which retained the ability to specifically recognize HLA-A2^{+} target cells pulsed with the mutant but not WT peptide. The genetic sequences encoding the α and β TCR chains from this clone were retrieved, and a reconstructed TCR using these sequences was cloned into a retroviral vector. Like the parental clone, T cells genetically engineered with the H3.3K27M-specific TCR acquired the ability to specifically recognize and kill target cells pulsed with mutant but not WT peptide.

Time and again, cancer immuno-therapists have unfortunately fallen prey to chasing peptide "ghosts": highly avid T cells which recognize peptide-pulsed targets that nevertheless fail to recognize HLA-matched, antigen express-ing tumor cells. Chheda et al. (2018) avoided this potential pitfall by providing multiple lines of evidence that their candidate epitope is processed, presented, and displayed on the surface of DMG cancer cells. First, the authors used a highly sensitive mass spectrom-etry-based assay (Bassani-Sternberg et al., 2016) to characterize the peptides bound to MHC molecules isolated directly from DMG cell lines. Using this technique, they identified a variant of the 10-mer mutant peptide only in cells harboring the H3.3K27M mutation but not cells with WT H3.3. Second, using a series of DMG cell lines that all possess the H3.3K27M mutation but are variable in HLA-A2 expression, the authors showed that T cells transduced with the H3.3K27M-specific TCR only recognize HLA-A2^{+} tumor cells. In an

elegant series of reversion experiments, the authors subsequently demonstrated that transduction of HLA-A2 into an HLA-A2- H3.3K27M[+] DMG cell line enabled T cell recognition while an anti–HLA-A2 antibody blocked recognition. Critically, additional experiments demonstrated that HLA-A2[+] tumor cell lines that were WT for H3.3 were not recognized. This suggests that engineered T cells might be able to distinguish between healthy tissues and tumor cells. Finally, the authors tested whether systemically administered T cells engineered with the mutation-specific TCR treat H3.3K27M^{+} tumor cells injected within the intracranial cavity of immune-deficient mice. Whereas tumor growth was unabated in mice receiving mock-transduced T cells relative to a saline control, animals receiving TCR engineered cells had a protracted arrest of tumor growth.

Collectively, these data describe an exciting and eminently translatable discovery of a novel "public" neoantigen (see figure). The H3.3K27M$_{26-35}$ epitope now joins the ranks of other recently uncovered "public" neoantigens resulting from hotspot mutations in driver oncogenes. For example, a series of immunogenic KRAS hotspot mutation–specific epitopes have also been reported which are restricted by HLA-C★08:02 and HLA-A★11:01 (Tran et al., 2016; Wang et al., 2016). Clinically, knowledge

of "public" neoantigens could be used to boost what appears in a limited number of tested patients to be a preexisting T cell response using various vaccination approaches. Indeed, this approach using an H3.3K27M$_{26-35}$ peptide vaccine in combination with the immune-adjuvant Poly-ICLC is now already under way (NCT02960230). It remains to be seen, however, whether any T cell vaccine is potent enough to induce cancer regression in patients with metastatic cancer even if this exceptional class of antigens is targeted (Klebanoff et al., 2011). As suggested by proof-of-concept experiments in the current manuscript, an alternative approach could be to genetically redirect a patient's T cells to recognize a "public" neoantigen through introduction of a TCR followed by adoptive T cell transfer. The first clinical trial to test this concept in HLA-A★11:01[+] patients with cancers harboring the KRAS G12V hotspot mutation has recently been initiated (NCT03190941), and it is possible that the TCR described by Chheda et al. (2018) may rapidly follow suit. Time will tell what the ultimate utility of "public" neoantigens will be. There are ample reasons to believe, however, that they will help democratize the potential benefits of targeting cancer neoantigens to a far greater number of patients more efficiently and rapidly than would otherwise be the case.

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