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The landscape of T cell antigens for cancer immunotherapy

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The remarkable capacity of immunotherapies to induce durable regression in some patients with metastatic cancer relies heavily on T cell recognition of tumor-presented antigens. As checkpoint-blockade therapy has limited efficacy, tumor antigens have the potential to be exploited for complementary treatments, many of which are already in clinical trials. The surge of interest in this topic has led to the expansion of the tumor antigen landscape with the emergence of new antigen categories. Nonetheless, how different antigens compare in their ability to elicit efficient and safe clinical responses remains largely unknown. Here, we review known cancer peptide antigens, their attributes and the relevant clinical data and discuss future directions.

Recent years have seen tremendous clinical benefit from cancer immunotherapy; however, although multiple immunotherapeutic modalities exist[1,2] (Box 1), these largely converge to cytotoxic T cells (CTLs) targeting the tumor (Fig. 1). T cells are activated through specific T cell receptor (TCR)–antigen interactions. V(D)J recombination can generate a huge diversity (up to ~10^{15}, theoretically) of T clonotypes in the thymus, each with its unique TCR (or two TCRs, in the case of αα/αβ clonotypes)[3]. This repertoire is further pruned by positive and negative selection processes, yielding ~10^6–10^{10} circulating clonotypes[3]. Each TCR can bind a particular (albeit unknown) set of antigens, thereby defining T cell specificity. T cell antigens are presented on two types of major histocompatibility complex (MHC) molecules, termed human leukocyte antigens (HLAs) in humans. MHC class I (MHC-I) molecules are expressed by all nucleated cells, whereas MHC class II (MHC-II) molecules are expressed by antigen-presenting cells (APCs), epithelial cells and some tumors[4]. Peptides presented on MHC-I originate intracellularly primarily as proteasomal degradation products and are recognized by $CD8^+$ CTLs, whereas the peptides that present on MHC-II are derived from exogenous or membrane proteins that are degraded by the endosomal/lysosomal system and recognized by $CD4^+$ T cells. Overriding this principle is the process of cross-presentation, whereby exogenously sourced peptides are presented on MHC-I mainly by $XCR1^+CD103^+$ type 1 dendritic cells (DC1s)[5], which then migrate to tumor-draining lymph nodes and prime T cells against tumor antigens[6]. Cross-presentation is crucial for $CD8^+$ T cell priming and the maturation of tumor-recognizing CTLs.

It is widely established that tumor immune-rejection is T cell-mediated and the antitumor T cell response is antigen-specific[7]. Objective tumor regressions following antigen-selective and TCR-engineered adoptive cell transfer (ACT) treatments support these assertions[8,9]. Genomic library screens have uncovered several prototypes of tumor-rejection antigens, including mutation-derived antigens (called neoantigens) and cancer germline antigens. The field has long recognized the archetypic distinction between tumor-specific antigens (TSAs), which are exclusively presented on tumor cells, and tumor-associated antigens (TAAs), which are also present on additional tissues. Advances in immunotherapy (Fig. 2), and in the methods available for T cell antigen identification[1,10,11] (Fig. 3), resulted in a surge of interest to identify and characterize tumor-presented T cell antigens, moving the field beyond classic TSA and TAA types, to previously unappreciated sources of cancer antigens, such as non-canonical and bacterial proteins[12–14]. The increased availability of sequencing data enabled the systematic exploration of cancer neoantigens, including the subgroup of recurrent (public) neoantigens[15].

Here we review the main classes of cancer T cell antigens known to date, discuss the attributes of effective cancer antigens and compare

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BOX 1

Immunotherapeutic strategies for induction and modulation of T cell responses

ACT includes the treatment of patients with their own, naturally occurring or genetically engineered tumor antigen-reactive T cells[208,209]. Typically, resected neoplastic tissue obtained through biopsy or surgery is used to extract TILs, which are then massively expanded ex vivo. In addition, TCRs recognizing tumor antigens can be isolated and endogenous T cells can be genetically engineered to express these TCRs.

The relative ease with which the antigen component may be adapted, thereby providing an avenue for personalized treatment, along with its straightforward administration, also makes vaccination an attractive strategy. Vaccination aims at de novo generation of tumor-antigen-specific T cell responses as well as augmentation of existing T cell responses by delivery of tumor antigens to professional APCs together with their proper activation via adjuvants. Evidence supporting the efficacy of vaccination in disease control is now emerging[210].

Another immunotherapeutic strategy approved in clinical practice is ICB, which unleashes the T cell potential against the tumor. The leading ICB targets, PD-1 and CTLA-4, are both inhibitory co-receptors whose expression is upregulated in T cells on antigen-dependent TCR stimulation. An analysis of human specimens revealed significant associations between increased $CD8^+$ CTL tumor infiltrates and response to PD-1 blockade[211]. The level of such infiltrates greatly increases in responders during treatment and correlates directly with reduction in tumor size. Expansion of T cell populations, broadening of the TCR repertoire and depletion of intratumoral regulatory T cells are also outcomes of clinical relevance with CTLA-4 blockade[212].

the different antigen classes with respect to these attributes. Finally, we summarize the clinical data that support the usefulness of each antigen class and give a critical overview of progress in the field.

Known classes of cancer-associated T cell antigens

T cell antigens that permit efficient disease targeting are classified as TAAs or TSAs, depending on whether they are tumor exclusive. In this section we discuss an alternative, partly overlapping classification that relies on the type of source protein. Accordingly, cancer antigens can be divided into self-antigens, originating from normal proteins that are differentially expressed in the tumor; genomic alteration-derived neoantigens, which are degradation products of tumor-specific mutated proteins; non-canonical antigens, arising from unannotated open reading frames (nuORFs), translation aberrations or post-translational modifications; and microbial antigens, derived from proteins encoded by tumor-infiltrating microbes such as bacteria and viruses (Fig. 4).

Self-antigens

The precursors of tumor-associated self-antigens are non-mutated proteins that exhibit differential expression patterns in tumors. For instance, glycoprotein 100 (gp100)[16], tyrosinase[17] and melanoma antigen recognized by T cells 1 (MART-1)[18] are tissue-specific antigens expressed in melanoma. Tebentafusp, an immune-mobilizing monoclonal T cell receptor against cancer consisting of a soluble gp100-specific TCR fused to an anti-CD3 effector molecule, which yielded clinical benefit in patients with uveal melanoma[19] and was approved by the US Food and Drug Administration (FDA), exemplifies the potential of self-antigen-targeting therapy. Given that tumors frequently share similar gene-expression patterns with their tissue of origin, the use of tissue-specific antigens in therapy is limited by the extent of collateral damage to the surrounding healthy tissue.

Cancer germline antigens represent another class of self-antigens that stem from proteins expressed only in germline tissues (fetal testes and ovaries) and trophoblast cells. Germline genes are epigenetically silenced by promoter methylation in most healthy tissues, excluding the immune sanctuaries of germ and placental trophoblasts. Yet, in many human cancers, promoter demethylation reactivates their expression. An analysis of 153 cancer germline genes showed their highest aberrant expression is in skin, lung, liver and brain cancers[20]. Complementary DNA expression library screens have greatly contributed to the identification of such antigens, including the X-chromosome-linked melanoma-associated antigen (MAGE) family of antigens and NY-ESO-1 (refs. 21–23). Cancer germline antigens are less affected by central immune tolerance than other types of self-antigens due to their unique expression pattern[24], which together with their high prevalence in patients, makes them highly interesting immunotherapy targets; however, their expression in tumors was found to be heterogeneous due to their locally varying DNA methylation status[25].

Genomic alteration-derived neoantigens

Mutation-derived neoantigens feature cancer-distinct sequence aberrations encoded by somatic point mutations, frameshifts or chromosomal aberrations. Non-synonymous mutations that result in aberrant proteins can lead to the generation of genuine TSAs if their degradation results in HLA-binding neopeptides. Being the most abundant and simple form of mutations[26], non-synonymous point mutations are currently the best studied mutation-derived neoantigen precursors. Single amino acid changes may either alter the immunogenicity of an HLA-binding peptide[27] or, if they occur in anchor positions, turn a non-binding sequence into an HLA-binding one[28]. Alternatively, a mutated amino acid could give rise to a new proteasomal cleavage site, thus allowing peptide processing and HLA loading[29].

Indirect but notable evidence of the pivotal role that neoantigens hold in immune-mediated tumor regression comes from the observed association between tumor mutational burden (TMB) and immunotherapy response. The fact that high TMB stochastically increases the chance of neoantigen formation together with the central role of T cells in mediating tumor regression both in immune-checkpoint blockade (ICB) and ACT, suggests the involvement of a neoantigen-driven T cell response. Independent studies, across multiple cancer types and immunotherapy modalities found improved clinical outcomes with increased TMB[30–37]. Recently, neoadjuvant anti-programmed cell death protein 1 (PD-1) administration to 12 patients with stage II–III mismatch repair deficient (high TMB) rectal adenocarcinoma achieved 100% complete clinical response, alleviating the need for standard chemoradiotherapy[38]; however, this association does not hold for all tumor types as shown in glioma, where TMB could not predict immunotherapy efficacy[39].

The advent of next-generation sequencing has allowed the systematic, unbiased survey of mutations from individual tumors[40]. These data, in turn, can guide antigen discovery[10,41–43] either through T cell-based assays or HLA peptidomics[1,10,11] (Fig. 3). Data generated from extensive whole-exome sequencing-based screening raise the possibility that tumor-infiltrating lymphocyte (TIL) reactivities against mutation-derived neoantigens exist in the majority of cancers, not only in tumor types known to be amenable to immunotherapy[1]. Yet, less than 2% of the screened mutations are recognized by T cells, and the associated neoantigens are individually unique to each patient

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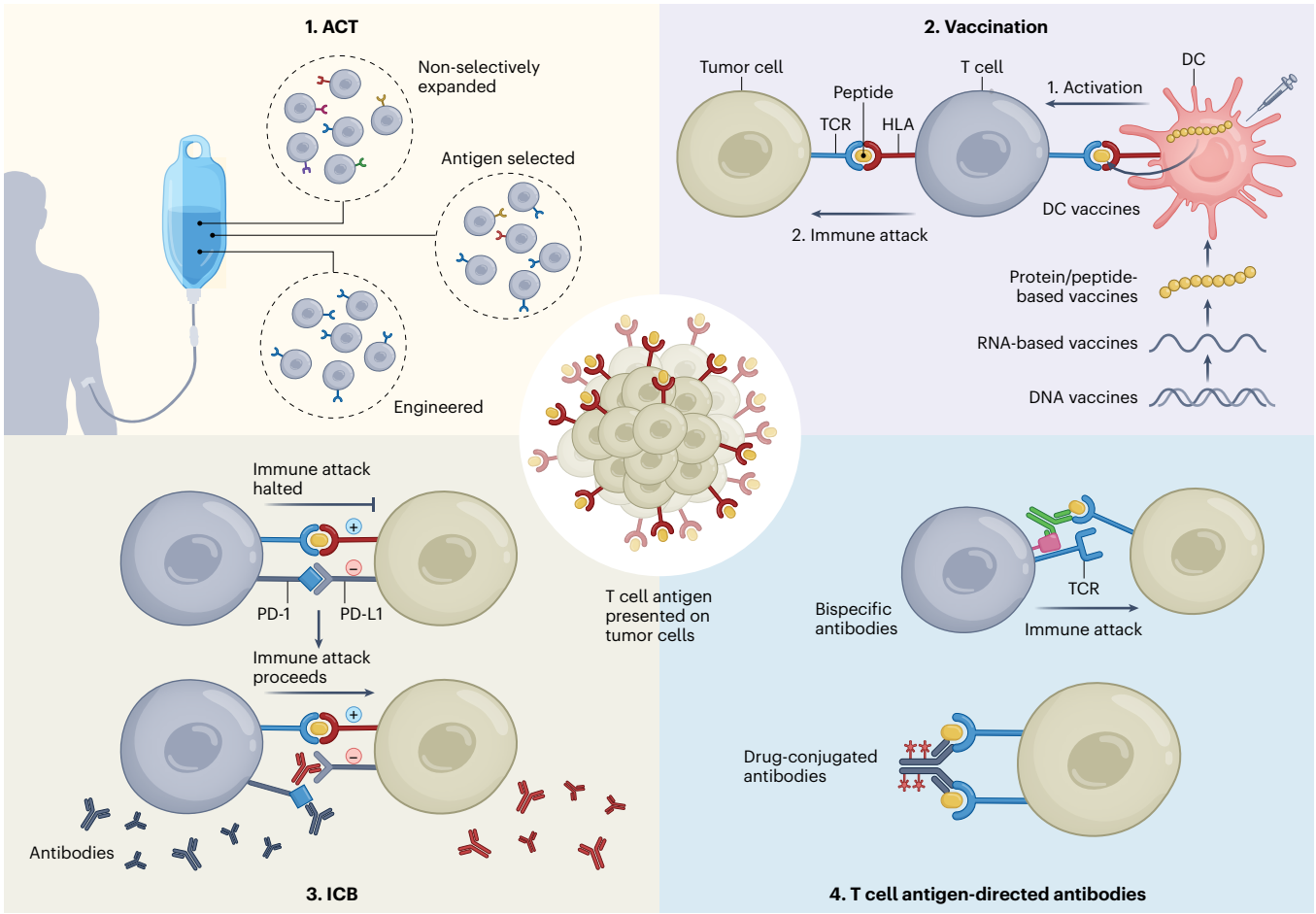

Fig. 1 | T cell antigens at the center of all immunotherapy modalities. Different immunotherapy modalities all converge onto T cell recognition of tumor antigens. (1) ACT of T cells specific for tumor cells according their TCR specificity. Patients are re-administrated with peripheral or intratumoral T cells previously isolated, ex vivo-expanded and, if applicable, TCR-engineered. (2) Vaccines of different types (for example, protein/peptide-, RNA- or DNA-based vaccine, DC vaccines) educate the immune system against specific tumor-presented antigens. Vaccines are taken up by local DCs that migrate to secondary lymphoid organs and prime and activate T cells that mediate tumor immune attack. (3) ICB unleashed pre-existing T cell–tumor interactions. Monoclonal antibodies specific for inhibitory receptors prevent ligation and unleash halted immune attacks. Anti-PD-1/PD-L1 therapy is illustrated, but mechanisms also hold true for other types of ICB. (4) Uni- and bispecific monoclonal antibodies that are directed at a specific T cell antigen on tumor cells may either recruit T cells for attach or may release a cytotoxic cargo. PD-L1, programmed cell death ligand 1.

(private neoantigens), greatly narrowing their applicability to the majority of patients[1].

In contrast, recurrent or public neoantigens derived from both point mutations and larger genetic aberrations, although scarce, have also been identified[27,44,45]. Unlike functionally unimportant private passenger mutations, driver mutations are functionally important and tend to be more clonal[46]. Finally, cellular therapies or vaccinations against recurrent mutations can benefit many patients with the same tumor type, but also patients with different cancer types harboring the same recurrent mutation.

Among the known neoantigens derived from recurrent mutations are CDK4.R24C[47–50], KRAS.G12V/C/D[51–53], EGFR[54–59] and PIK3CA.H1047L[60]. HLA peptidomics coupled with whole-exome sequencing[61] to survey the landscape of recurrent neoantigens in melanoma discovered an (N)RAS.Q61K/HLA-A*01:01-derived neoantigen that elicits T cell reactivity and cross-reacts with the highly prevalent (N)RAS.Q61R variant[27], suggesting that patients with RAS.Q61 mutations and HLA-A*01:01 could benefit from cellular treatment. A similar methodology is mutation-associated neoantigen selected reaction monitoring (MANA-SRM), an optimized immunoprecipitation and mass-spectrometry protocol for the detection of low-abundance neoantigens that was used to uncover several RAS- and IDH2-derived recurrent neoantigens[62]. To date, the most clinically promising result for recurrent neoantigen targeting was achieved with the $KRAS^{G12D}$ mutation. Two patients, one with metastatic colorectal cancer and the other with metastatic pancreatic cancer, harboring this mutation on HLA-C*08:02, were successfully treated with TCR-transduced T cells against the mutation[8,63], demonstrating the applicability of targeting recurrent neoantigens across different tumor types.

Less frequent types of mutations, such as insertions/deletions (indels), translocations and inversions, may also give rise to neoantigens. Frameshift indel mutations were significantly associated with response to anti-PD-1 or anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) in an analysis of three independent melanoma cohorts[64]. Further, the analysis indicated that frameshift mutations form a potentially more potent neoantigen landscape than an equivalent number of non-synonymous single-nucleotide variations (nsSNVs)[64]. In mesothelioma, a cancer that generally exhibits low mutational burden but a high degree of large chromosomal rearrangements, the inter- and intra-chromosomal rearrangements were predicted to bind HLA molecules and were recognized by patient autologous TILs[65].

Finally, fusion genes, such as the BCR–ABL fusion in leukemia (Philadelphia chromosome)[66] and the EML4–ALK[67] fusion in non-small cell lung cancer (NSCLC) have been shown to generate T cell

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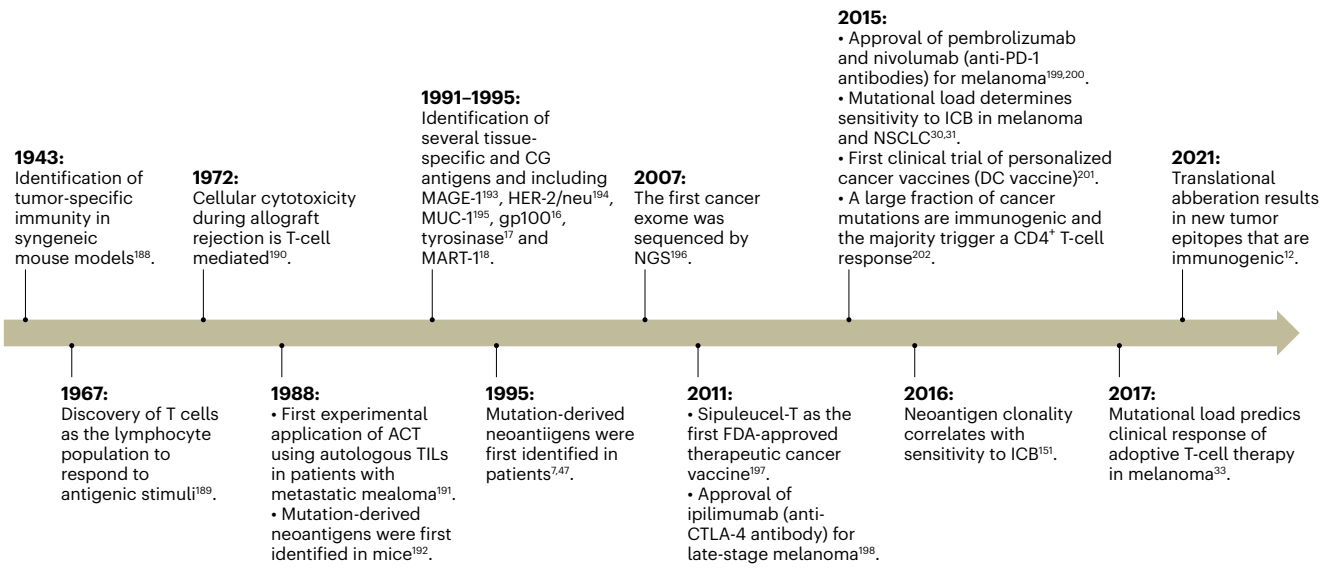

Fig. 2 | Advances and discoveries in tumor antigen research. A selection of seminal findings in tumor antigen research is highlighted. CG, cancer germline; MUC-1, mucin-1; NGS, next-generation sequencing. The following references are cited in the timeline: refs. 7,12,16–18,30,31,33,47,151,188,189,195–207.

recognizable neoantigens[68,69]. Although large-scale structural variations (deletions, duplications, inversions and translocations) occur frequently and are a potential source for tumor neoantigens, existing tools for their prediction from whole-genome sequencing lack sensitivity, thereby limiting their usefulness[70,71]. An analysis of RNA-sequencing data from 9,624 TCGA samples across 33 cancer types, using the STAR-Fusion, Breakfast and EricScript algorithms for fusion calling, identified 25,664 fusion events[72]. Across the different cancer types, 1.5 neoantigens were predicted per fusion using NetMHCpan 4.0 (ref. 73). In contrast, analysis of two cohorts of patients with melanoma treated with ICB did not find fusion gene scores to positively correlate with survival, whereas the overall neoantigen score (nsSNVs, indels and fusion genes) did, raising questions regarding the role of fusion genes as tumor-rejection antigens[68].

Tumor antigens from non-canonical transcriptional and posttranscriptional aberrations

Accumulating evidence suggests that noncoding gene translation frequently occurs[74] and that antitumor immune responses can be directed against tumor antigens derived from noncoding regions[75–77]. By combining HLA peptidomics, RNA-sequencing and ribosomal-sequencing data[77], hundreds of shared and tumor-specific non-canonical HLA-presented peptides stemming from lncRNAs, pseudogenes, transposable elements, untranslated regions (UTRs) of coding genes and alternative open reading frames were uncovered. Yet, of the >500 antigens screened for immunogenicity, only one was recognized by autologous TILs and peripheral blood mononuclear cells. The low expression of non-canonical antigens and, hence, their limited availability for in vivo cross-priming might underlie the low de novo T cell responses detected. Antigen-specific T cell responses have furthermore been observed against the intronic sequence *N*-acetylglucosaminyltransferase V gene (expressed in 50% of melanomas but not in healthy cells[75]), an incompletely spliced intronic region of gp100 (ref. 78) and the 5′ UTRs of c-akt oncogene[79]. Examples for immunogenic, MHC-presented peptides arise from alternate reading frames include NY-ESO[80], human epidermal growth factor receptor 2 (HER2), telomerase reverse transcriptase, prostatic acid phosphatase and nuORFs with non-AUG translation initiation sites[81–83]. Some nuORF neoantigens, such as the one stemming from a CUG-start-codon vascular endothelial growth factor, were found to be cancer-specific, whereas others are also expressed in healthy tissue[82,83].

Translational reprogramming and impaired translational fidelity in cancer cells can give rise to non-canonically translated peptides and potentially, new immunogenic antigens[84]. Such neoantigens arise from translation malfunctions due to ribosome frameshifting during amino acid deprivation[12], oxidative stress[85,86] or codon misreading by deregulated transfer RNAs[87]. Specifically, tryptophan-shortage-induced ribosome frameshifting[12] was shown to lead to the presentation of new transframe peptides on HLA molecules that are able to prime naive T cells. Notably, patient samples with shared HLA alleles harbored identical frameshifting-aberrant peptides, suggesting that these peptides may be a recurring cancer feature. Aberrant protein translation by amino acid deprivation are likely dynamic processes that depend on the tumor microenvironment and inflammatory and nutritional status, which puts into question their suitability for inducing antitumoral responses.

Finally, post-translational modifications (PTMs) can become deregulated in cancer cells, resulting in growth advantages[88] but also offering potential targets for cancer immunotherapy[89]. PROMISE, a computational pipeline for the detection of PTMs without enrichment has been used to identify numerous modified MHC-bound peptides with cancer-specific expression and their capacity to elicit a T cell response[89]; however, it is yet to be determined whether these PTM-derived antigens can elicit meaningful T cell responses for future cancer therapeutics.

Pathogen-derived tumor-associated antigens

Pathogen-derived TAAs are remnants of bacterial or viral infections. If acute infections are not cleared properly, viruses can remain inside host cells and (due to the expression of oncogenic proteins, induced immunosuppression and disruption of the host genome) mediate a malignant transformation (reviewed previously[90]). Pathogens that can directly drive cancer include *Helicobacter pylori*, which induces gastric cancer[91], human papilloma virus (HPV), which induces genital and head and neck cancers[91] and hepatitis B and C viruses (HBV and HCV), which cause hepatocellular carcinoma[92], among others. The reported de novo T cell responses against such pathogens[92,93] make inducing a specific T cell response against pathogen-derived antigens

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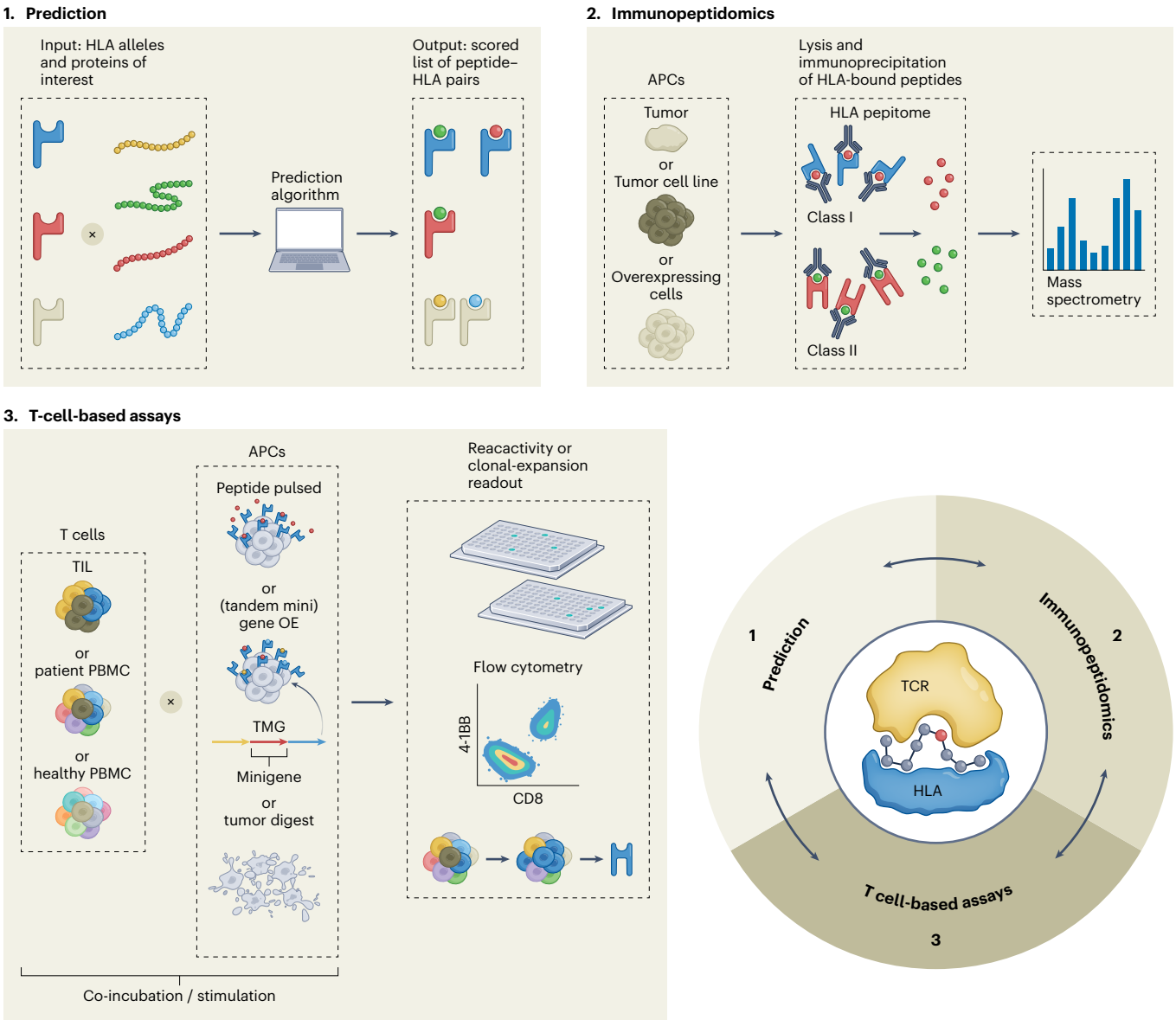

Fig. 3 | Methods for the identification of cancer T cell antigens. The three main routes for antigen discovery: (1) prediction, (2) immunopeptidomics and (3) T cell-based reactivity/expansion assays. Prediction algorithms (1), such as the popular NetMHCpan, receive as input protein/peptide sequences and a list of HLA alleles of interest and provide as output peptide/HLA pairs that are predicted to bind. In immunopeptidomics (2), HLA-peptide complexes are immunoprecipitated from cell lysates and then the peptides are eluted and analyzed by mass spectrometry to determine their sequences. This allows unbiased inquiry of the presented peptidome, regardless of immunogenicity. T cell-based assays (3) rely on in vitro co-incubation of T cells with presenting cells that express the protein–HLA pairs of interest. Reactivity or expansion readouts are then utilized to confirm antigen presentation. An added advantage with this approach is that antigen-reactive T cells may be isolated and further utilized. The flow of information between these methods aids in their improvement. Validated antigens from immunopeptidomics and T cell-based assays are used to train better prediction algorithms. Predictions, in turn, serve to narrow the search space in immunopeptidomics or T cell-based screens and add credibility to identified hits. OE, over expressed; PBMC, peripheral blood mononuclear cell.

a promising strategy to elicit immune responses against cancer cells, while sparing healthy tissue that lacks pathogenic antigen expression. This may be achieved through therapeutic cancer vaccines[94,95] or ACT[96].

For instance, peptides from different intratumoral bacteria were found to be presented on patients' HLA molecules and trigger antigen-specific immune responses in melanoma[13]. Accordingly, the knockout of β-microglobulin (B_2M) or MHC-II transactivator (CIITA) caused a decrease in the number of HLA-I and HLA-II presented bacterial peptides. Antigens derived from other types of microbiome, such as the virome, may emerge either with an intrinsic ability to elicit T cell responses or to cross-react with other TAAs in a form of molecular mimicry. An example for this concept is the prophage-encoded antigen TMP1, which activates T cells that are reactive against PSMB4. The *Enterococcus hirea* strain 13144 carries the phage and is abundant in lung an renal cancers, with the presence of the prophage in human patients correlating with response to immunotherapy[97]. Another possible source for viral element-derived antigens is the human endogenous retrovirus ERVE-4 whose expression was associated with immunotherapy response in clear cell renal cell carcinoma[98]. Common oncogenic viruses have the potential to form widely applicable T cell targets if the processing of persistently expressed oncoproteins intersects with prevalent HLA binding. High-risk HPV strains are involved in ~5% of all

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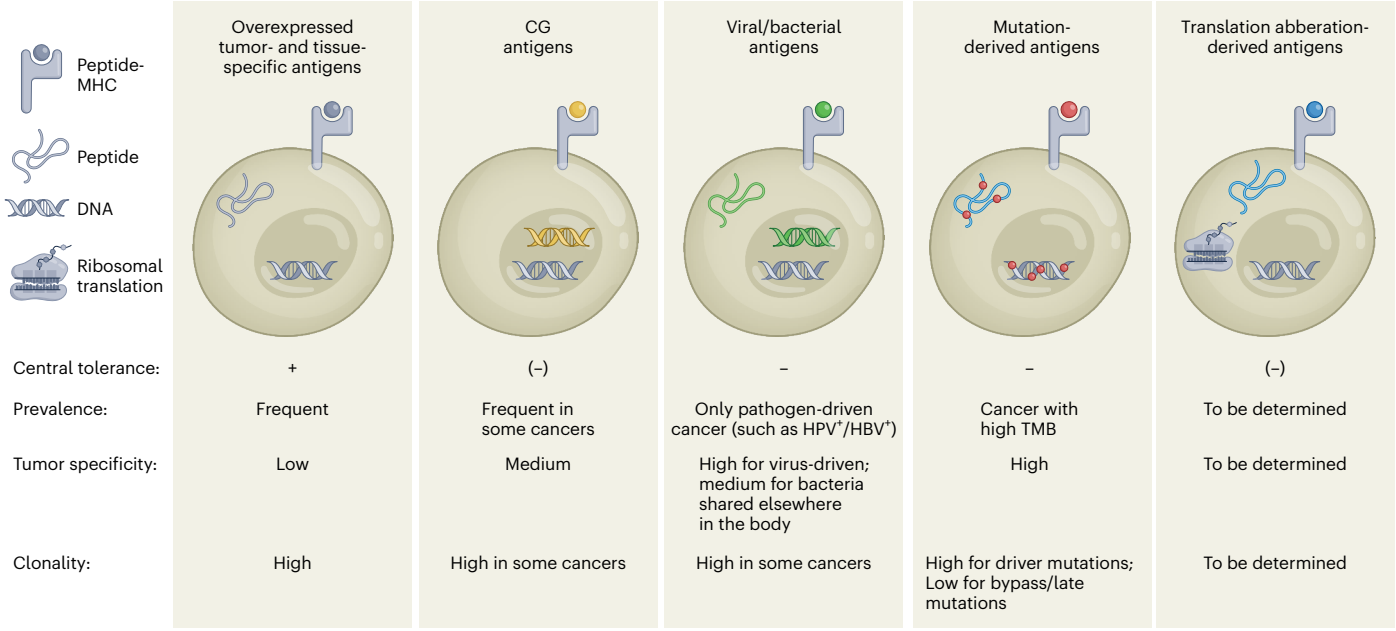

Fig. 4 | Tumor antigens recognized by T cells. Different classes of TSAs, their potential to evade central tolerance, prevalence, tumor specificity and clonality. Overexpressed tumor- and tissue-specific antigens are ubiquitously present in tumor cells; however, they are shared with healthy tissues and thus have low tumor specificity and are hampered by central tolerance. CG antigens are solely expressed in the germline and become re-expressed in tumor cells, providing them with a medium tumor specificity and subjecting them to central tolerance. Viral and bacterial antigens stem from former oncogenic pathogen infection, which renders them highly tumor-specific with no expression in healthy tissue and a lack of central tolerance. Neoantigens arising from mutation, for example single-nucleotide variations, indels or fusion genes arise from oncogenesis and are exclusively present in cancer cells, harbor a high tumor specificity and no central tolerance. Aberrant translation-or transcription-derived neoantigens are a result of malfunctional cellular transcription and translation machinery in cancer and are not encoded by the genome. As a rather new class of TSAs, their prevalence and tumor specificity largely remain to be explored.

human cancers, in particular, cervical and oropharyngeal malignancies. Moreover, immunogenic peptides have been identified deriving from the HPV-related cancer driver genes E6 and E7 that are restricted to the highly prevalent HLA-A*02:01 alleles[99,100]. Finally, the recent discovery of fungi in various tumors with distinct compositions[101] may suggest that fungi-derived antigens, whether they exist and able to elicit T cell reactivity, could be another layer of tumor antigens.

Tumor antigen attributes contributing to antitumor immunity

The antitumor potency of any given antigen relies on a combination of attributes, some of which are unique to T cell targets (for example, immunogenicity and effective cross-presentation), whereas others would be applicable to any form of targeted therapy (for example, population-wide prevalence, disease specificity, clonality and functional significance). Determining the optimal combination of features is not a trivial task. For example, mutation-derived neoantigens are immunogenic but tend to be private, whereas self-antigens are widely applicable, but less immunogenic. Accumulating clinical experience provides invaluable insight into the usefulness of self-antigens and mutation-derived neoantigens; however, the therapeutic potential of newly explored antigen classes, such as non-canonical neoantigens and bacterial antigens remains unclear. In this section we discuss the various properties contributing to the therapeutic effectiveness of an antigen in terms of four main parameters: the prevalence of each antigen category in the patient population, the specificity of each category to tumor cells rather than somatic cells, the immunogenicity and clonality of different antigens (Fig. 4).

Population-wide prevalence

The population-wide prevalence of an antigen is a strong determinant of its therapeutic utility. Some cancer antigens, such as the prototypic TSA exhibit high recurrence rates. Cancer germline antigens display differential frequencies across tumor types and disease stages. The cancer germline protein MAGE-A1, for example, is observed in less than 20% of primary malignant melanomas, 48% of metastatic melanoma cases, 25% of ovarian cancers, but only in 3.5% of leukemias[102]. Recurrent neoantigens derived from KRAS, NRAS, TP53, PIK3CA and BRAF are expected to be relevant to thousands of cancer patients yearly[15,27,60]. Notably, as in all T cell antigens, therapeutic targeting of recurrent neoantigens depends on specific HLA-peptide composition, which requires the combination of both a highly prevalent HLA and peptide.

The prevalence of non-canonical neoantigens ultimately depends on the robustness of the underlying generative process and on the ubiquity of the precursor protein across tumors. nuORFs originating from tissue-specific, cancer germline or overexpressed transcripts may potentially be as prevalent in the population as their canonical counterparts[76,78,80]. In an analysis of ten tumor samples from different patients, about half of the detected nuORFs were shared between at least two samples, suggesting that nuORFs are valid precursors for recurrent antigens[14]. The same study validated two nuORF melanoma antigens whose source genes are highly overexpressed in 28% (a pseudogene) and 59% (lncRNA) of TCGA melanoma samples.

Recurrence of ribosomal frameshifting-derived neoantigens has also been reported. Specifically, tryptophan-shortage-induced ribosomal frameshifting in melanoma cells has been linked to prolonged interferon (IFN)-γ exposure[12], and immunopeptidomics of IFN-γ-treated tumor samples with shared HLA alleles has revealed recurrent mis-translated peptides. Finally, the extent of pathogen-derived cancer antigens is currently unknown and should be addressed in future studies. As every cancer type is characterized by its own unique microbial repertoire[103], the full landscape of bacterial antigens is likely to be immensely diverse, and together with viral and even fungi-derived antigens, remains to be elucidated.

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Table 1 | Selected published clinical trials utilizing self-antigens as targets

Year of publication	Investigator /sponsor	Clinicaltrials.gov identifier, phase	Indication	Platform /treatment	Key results	Ref.
2006	NIH	NA, NA	Metastatic melanoma	Adoptive transfer of MART-1 specific TCR-engineered T cells	12% of patients experienced tumor regression	107
2008	Fred Hutchinson Cancer Research Center	NA, NA	Metastatic melanoma	Adoptive transfer of NY-ESO-I specific $CD4^+$ T cells isolated from blood	Complete regression on a single patient (case report)	158
2009	NIH	NCI-07-C-0174, 1	Metastatic melanoma	Adoptive transfer of MART-1 and gp100-specific TCR-engineered T cells	30% and 19% of patients receiving MART-11 or gp100 TCRs, respective experienced objective antitumoral response along with toxicity	104
2011	NIH	NCT00923806, 1	Metastatic colorectal cancer	Adoptive transfer of CEA specific TCR-engineered T cells	All patients experienced decreases in serum CEA levels with one patient with objective regression along with toxicity	157
2011	NIH	NCT00670748, 1	Metastatic synovial cell sarcoma and melanoma	Adoptive transfer of NY-ESO-I specific TCR-engineered T cells	~50% of patients with each indication experienced objective clinical responses	159
2014	Jonsson Comprehensive Cancer Center	NCT00910650, 2	Metastatic melanoma	Combination of MART-1 peptide pulsed dendritic cell vaccine and adoptive transfer of MART-1specific TCR-engineered T cells	69% of patients experienced tumor regression	162
2015	Adaptimmune / GSK	NCT01352286, 1/2	Multiple myeloma	Adoptive transfer of NY-ESO-I and LAGE-1 specific TCR-engineered T cells	80% of patients experienced objective clinical response	161
2017	NIH	NCT02111850	Metastatic cervical, urothelial, esophageal cancer, osteosarcoma	Adoptive transfer of MAGE-A3 specific TCR-engineered $CD4^+$ T cells	25% of patients expressed regression of tumors, one cervical cancer patients exhibiting complete response	163
2018	Adaptimmune/GSK	NCT01343043, 1	Metastatic or recurrent synovial sarcoma	Adoptive transfer of NY-ESO-I specific TCR-engineered T cells	~50% of patients experienced objective clinical responses	109
2020	BioNTech/TRON	NCT02410733, 1	Metastatic melanoma	Systemic mRNA vaccine encoding for multiple self-antigens	A majority of patients experienced regressions in multiple target regions	165

NIH, National Institutes of Health; NA, not available.

Tumor specificity

For a cancer treatment to be tolerable, cytotoxicity must be confined to the tumor and must be considerably less abundant in healthy tissue. Despite other fundamental advantages, shared TAAs fall short in this respect. Tissue-specific antigens derived from MART-1 and gp100 resulted in disease regression in 30% and 19% of melanoma patients, respectively, but also in significant immunotoxicities[104]. Lethal cardiac toxicities and cytokine release syndrome from on-target anti-MART-1 effects have been documented[105,106]. Conversely, toxicity resulting from the gp100 targeting soluble TCR product tebentafusp, used for uveal melanoma, is reasonably tolerated[19]. Keeping in mind the small scale of these trials, it should be noted that although a less-avid anti-MART-1 TCR produced a weaker therapeutic effect (12% of patients exhibited tumor regression), it also did not induce toxicities[107]. In contrast, the unique expression pattern of cancer germline antigens should in principle make them practically tumor specific. Not surprisingly, this class dominates TCR-T clinical trials, with NY-ESO-1 the most targeted antigen precursor (Table 1), with NY-ESO-1 TCR-T showing promise as both an effective and tolerable treatement[108,109]. In practice, some cancer germline antigens do present outside of immune sanctuaries and in amounts that can induce fatal toxicities. In an anti-MAGE-3 TCR-T trial, for example, cross-reactivity toward MAGE-12 in the brain caused severe neurological sequela in three patients, resulting in the death of two of them[110].

As they derive from somatic mutations that accumulate during tumorigenesis, neoantigens are the epitome of TSAs. One major concern when targeting neoantigens is cross-reactivity toward the wild-type variant. The majority of discovered neoantigens exhibit point mutations at the TCR-exposed region of the neopeptide. Therefore, their HLA-anchoring region is expected to be similar to those of HLA complexes in healthy tissues[111]. In this regard, frameshift-derived neoantigens are potentially superior to point-mutation neoantigens. Nevertheless, direct comparisons of mutant versus wild-type TCR reactivity frequently revealed sufficient mutant specificity (no observed wild-type reactivity even at supra-physiologic peptide concentrations)[27,60,112]. An understudied neoantigen-related concern is the prevalence of somatic driver mutations in aging healthy tissues. The deep sequencing of non-cancerous esophageal and skin samples revealed a notably high burden of cancer-associated mutations[113,114], with TP53 found to be mutated in ~37% of healthy esophageal epithelium. Thus, caution is required even when targeting otherwise promising hotspot-derived neoantigens.

Although the disease specificity of non-canonical antigens remains unexplored, it likely depends on the underlying process that generates them. For example, cancer-associated chromosomal abnormalities might increase the proportion of new protein isoforms specifically in the tumor[115]. Similarly, overexpressed and cancer germline precursor

genes may yield differentially expressed non-canonical antigens even if the underlying generative process is not differentially activated in cancer cells[14,77,89]. Factors in the tumor microenvironment, such as IFN-γ produced under inflammatory conditions, can potentiate local generative processes such as ribosomal slippage events or single amino acid substitutions[12,116]. Whether non-canonical cancer antigens are therapeutically tolerable requires further investigation.

Oncogenic viruses exhibit tropism toward the tissues in which they drive oncogenesis. High-risk HPV strains, for example, mainly infect the mucosal epithelium of anogenital tissues (cervix, vagina, vulva, anus and penis) and the oropharynx. Hepatitis viruses chronically infect the liver. Epstein–Barr virus is maintained in epithelial cells of the pharynx, B cells and natural killer cells. MCPyV infects skin cells[117]. Differential tropism likens viral antigens to tissue-specific or shared antigens and if extratumoral infection is not widespread, off-tumor effects may be tolerable or at least manageable. For example, although non-cancerous liver tissue destruction is a concern with anti-hepatitis ACT, using it in liver transplant patients may be a viable option[118], given that ACT products that contain antiviral specificities are considered safe[100,118–122]. Nevertheless, viral occupancy in healthy tissues has not been sufficiently studied. For example, HCV RNA and antigens were detected extrahepatically in the kidney, heart, pancreas, intestine, adrenal gland, lymph nodes and gallbladder of HCV-infected cadavers[123]. In the case of bacterial cancer antigens, it seems that the cancer microbiome is cancer type-specific, resulting in differential tropism depending on the cancer type; however, tumor and immune cells of the tumor microenvironment are infected by bacteria[103]. Moreover, the detected bacterial genera are not rare in non-cancerous tissues and differential infection or antigen expression in tumor versus healthy tissues has not yet been established[13].

Antigen immunogenicity

The immunogenicity of antigens can be broken down into three main variables: (1) the functional avidity of reactive T cells to a certain antigen; (2) the antigen level of expression or cell-surface density on tumor cells; and (3) its effective cross-presentation by DC1 cells taking up tumor material. Notably, immunogenicity is best regarded as a potential rather than a constant trait. It is highly affected by the antigenic and inflammatory context within which the antigen is being presented and not an antigen autonomous trait. The importance of antigenic context is exemplified by domination of T cell reactivities toward a handful of antigens and as discussed below, by clonal fraction.

Functional avidity is most commonly assayed in vitro by cytokine release, upregulation of activation markers or target cell lysis at varying peptide concentrations. Multiple factors affect functional avidity, including TCR affinity (measured as K_d, the ratio of the association and dissociation rates, k_{off}/k_{on}), cell-surface density and organization (which contribute to TCR avidity) and the functionality of co-stimulatory interactions (for example, CD8:HLA, CD80/86:CD28 and ICAM-1/LFA-1) and intracellular signaling pathways. Effective T cell triggering is thought to require mid-range affinity to allow both serial engagement and sufficient dwell time[124,125]. Although low-affinity TCRs will not induce adequate activation, affinities that are too high may result in anergy or deletion[126]. A K_d of ~5 μM has been proposed as a TCR affinity threshold, above which $CD8^+$ T cell function cannot be improved[127]. Moreover, according to a recent simulation analysis, TCRs with equal affinity may differ in the functional avidity they confer even when all other parameters are equal. Therefore, alternate (k_{on}, k_{off}) formulations should be considered to predict functional avidity[128].

Not surprisingly, lower affinity/avidity values are observed against tumor-associated self-antigens compared to viral antigens or neoantigens[129,130]. On average, TCRs against tumor-associated self-antigens have ten-times lower binding affinities than those against viral antigens (100 μM versus 10 μM, respectively)[130]. A K_d of ~10 μM has been suggested to best balance antitumor efficacy and autoimmune risk for tumor-associated self-antigens[131]. The unique expression pattern of cancer germline antigens predicts that they will be less affected by negative selection than other self-antigens; however, this assertion has not been studied systematically. Evidence from a mouse model supports the lack of central tolerance against cancer germline antigens and a naturally occurring anti-NY-ESO TCR (1G4) exhibits a K_d of 11 μM, which is a low value for self-antigens[24,130]. Nonetheless, evidence exists for at least some level of expression of cancer germline antigens in the thymus[130].

As opposed to the low affinity of TAAs, nonself-antigens (be they canonical, non-canonical or pathogen-derived) are not being tolerized during thymic T cell repertoire development and thus are predicted to have higher affinity. Although TIL reactivities against bacterial and non-canonical specificities have been observed, the quality of these interactions has not been sufficiently characterized. As foreign entities, one would assume high TCR efficiencies for bacterial tumor-presented antigens. Non-canonical peptides that are truly aberrant, and do not present in the thymus, may also be exempt from central negative selection. Finally, high-affinity/avidity TCRs can be engineered even for non- or weakly immunogenic antigens, using humanized model species through vaccination or in vitro optimization strategies[104,132,133]. Targeting T cell antigens with engineered antibodies or chimeric antigen receptors is a viable option, as highlighted by preclinical studies[134–137], as is using engineered TCRs, exemplified by a $KRAS^{G12D}$-directed TCR on the HLA-A*11:01, which was engineered to have a 10^6 higher affinity compared to the original naturally occurring TCR[138].

Apart from affinity, TCR signaling depends on the number of TCR–pHLA (peptide–HLA) interactions at equilibrium; a function of both the affinity of the interaction (as discussed above) and the density of pHLA ligands on the cell surface. This is a complex interplay: although long TCR-–pMHC half-lives (TCR dwell time) result in impaired T cell activation for low pMHC densities, these are non-restrictive at high antigen densities[139]. pMHC antigen density depends on the expression level of the precursor protein, its degradation rate and the affinity of the peptide–HLA interaction itself. Sufficient presentation on APCs (for example, cross-presentation) is required for T cell priming, whereas the amount on tumor cells is important for their proper killing. High pHLA affinity contributes to both these processes, with an estimated threshold of 10 nM required for tumor eradication[140,141].

In tumors, endogenously expressed HLA-I TAAs (derived from NY-ESO-1 and MAGE-1) were detected at ratios of 10–150 copies per tumor cell using soluble TCR probes[142]. Quantitative immunopeptidomics reports indicate a wide range of tumor antigen densities[62,143]. This variation is because different peptide antigens from the same TAA precursor (PMEL) could have an order-of-magnitude difference in their number of presented copies in the same tumor cell line[143]. Based on the quantification of multiple cancer cell lines, neoantigens seem to present a few to several dozen copies per cell[62,134]. Many non-canonical proteins are defective, unstable and short-lived[144]. Owing to their rapid degradation, they are estimated to generate MHC-I peptides fivefold more efficiently per translation event[115]; however, to the best of our knowledge, the copy numbers that such individual antigens contribute has not been estimated[14,115]. Intracellular viral and microbial pathogens have evolved molecular mechanisms to decrease the presentation densities of their derived antigens[139,145] and thus might represent less ideal therapeutic targets.

Although direct HLA-I presentation on tumor cells is enriched for short-lived, rapidly degrading proteins[144,146], efficient cross-presentation requires the sufficient transfer of precursor proteins into presenting cells, thus favoring long-lived, stable, highly expressed substrates[147]. Dampened cross-presentation of unstable proteins has been postulated to effectively diminish the contribution of non-canonical antigens to antitumor immunity[144]. In a mouse model,

insufficient cross-priming by a lowly expressed tumor-rejection antigen precluded tumor regression, despite adequate presentation on tumor cells[148]. This could be ameliorated by therapeutic enhancement of cross-priming through vaccination or by anti-CD40 administration. Such interventions mark an untapped opportunity to harness non-canonical and lowly expressed antigens (which are usually not considered for vaccine design) for cancer therapy.

Antigen clonality

In addition to the amount of a presented antigen, its distribution across cancer cells (its clonality) is also critical. As intratumor heterogeneity alongside TMB are important determinants of antitumor immunity and responsiveness to immunotherapy[149], the immune system's ability to detect and eliminate antigen-bearing cells depends on their clonal fraction within the tumor[150]. Sub-clonal antigens that present on only a fraction of tumor cells are thought to facilitate tumor escape through the outgrowth of antigen-deficient cells. Thus, achieving effective immune control across all sites of a metastatic disease by targeting sub-clonal antigens is less likely. Indeed, the burden and fraction of clonal neoantigens correlate with response to ICB in lung cancer and melanoma[151]. Furthermore, in a mouse model of controlled intratumor heterogeneity, mixing together immune susceptible clones resulted in a polyclonal, immune-resistant tumor[152].

Tumor antigens vary substantially with regard to their clonality. Although self-antigens are generally considered clonal, the expression of cancer germline antigens within tumors was found to be heterogeneous due to locally varying DNA methylation status[25]. Linked to clonality, the essentiality of the precursor protein to cancer survival also bears significance as reliance on functionally unimportant proteins facilitates evasion through elimination or downregulation of the protein[153]. Although most mutation-driven neoantigens stem from passenger mutations, the subgroup of recurrent (public) neoantigens has the added value of functional relevance, and oftentimes also of clonality, making them superior therapeutic targets; however, evasion might ensue even when targeting clonal antigens that derive from functionally important proteins. For example, after initial regression of colorectal cancer metastases following KRAS-directed ACT, one metastasis recurred with HLA haplotype loss[8]. In another study, the metastasis of a primary tumor bearing an immunogenic BRAF neoantigen showed no trace of this oncogenic mutation in sequencing analyses[58]. Such reports exemplify that, much like with targeted therapies, and regardless of antigen clonality, combinatorial approaches to target multiple antigens, at once or sequentially, should be considered when possible.

Single-cell dissection of TIL specificities enables comparison of antigen classes

Coupled TCR and RNA single-cell sequencing have been used to map TIL specificities and transcriptional phenotypes in melanoma, lung, breast, colon and rectal cancers[129,154–156]. Two of these studies showed that tumor-reactive cells occupy shared exhaustion/dysfunctional states regardless of their target antigen class[129,154]. Furthermore, TILs targeting neoantigens and self-antigens could not be transcriptionally differentiated[129]. Conversely, bystander lymphocytes, including clones that target viral antigens, gravitated toward effector memory or tissue-resident memory phenotypes[129,156]. Prospective reactivity testing of TILs matching a neoantigen-reactive transcriptional program identified, in addition to neoantigen-specific cells, viral and shared-antigen specificities, as well as tumor-reactive orphan receptors[154]. Unlike bystander antiviral cells, the viral-specific clone in this case targeted an HPV-derived antigen and originated from an HPV-positive tumor. Transcriptomic patterns enriched for neoantigen-specific T cells correlated with pathologic response to ICB, indicating the functional importance of these newly defined phenotypes[156]. The above-mentioned work therefore marks different transcriptional programs for tumor-targeting versus non-tumor-targeting T cells but does not find distinguishing patterns between tumor-targeting T cells of different antigen classes. It is possible that functionally important differences would emerge when directly contrasting tumor-reactive clonotypes of different antigens classes; however, such analyses would require larger scale knowledge of TIL specificities.

Owing to the apparent transcriptional common ground of tumor-reactive TILs, one may expect immunomodulating approaches, such as ICB, to affect tumor-reactive T cells in a similar manner, regardless of the antigen class that they target. How this modulation plays out may depend on additional factors, such as TCR avidity. Higher TCR avidities have been observed for neoantigens compared to self-antigens, as would be expected from differential thymic selection pressure for these two types of antigens[129]. Another study pointed to functional avidities of neoantigen-reactive TCRs that are on par with those of antiviral TCRs[156], noting markedly higher neoantigen-specific avidities in ICB major pathologic responders (MPRs) compared to non-MPRs. Notably, many tumor-reactive exhausted cells in these studies bear orphan TCRs. While some of them probably target overlooked self, viral or mutation-derived neoantigens as demonstrated elsewhere[156], we speculate that full delineation would reveal specificities also toward less-studied classes of cancer-presented antigens, such as non-canonical neoantigens and bacterial peptides.

Therapeutic utility of T cell antigens

The choice of antigen(s) is of utmost importance for the success of antigen-directed immunotherapies. Although arguments can be made as to the merits and drawbacks of each antigen class, one should focus on the accumulating clinical data alluding to their therapeutic efficacy. Self-antigens and mutation-derived neoantigens are the two antigen classes that have been studied most extensively, with demonstrated contributions to immunomediated tumor control (Tables 1 and 2). Attempts have also been made to harness antiviral T cell responses for cancer control (Table 3). Newer antigen classes such as non-canonical and bacterial-derived peptides are yet to be tested. In this section we discuss the implications of key clinical findings relating to the different antigen classes.

Clinical experience with self-antigens

As discussed above, the prevalence of self-antigens in healthy tissues impacts both the efficacy and safety of treatment. The difficulty of balancing tolerance against these antigens (which must be overcome to reach clinical benefit) and excessive immune response (which could manifest in severe toxicity) leads to mixed results in clinical trials (Table 1). TCR-T therapy against a melanocyte-specific MART-1/HLA-A*02 antigen induced objective cancer regression in 12% of patients with melanoma[107]. Higher-avidity TCR (DMF5) induced an improved response rate (30%), along with significant toxicities to normal melanocytes[104]. Similarly, targeting of gp100- and carcinoembryonic antigen (CEA)-derived tissue-specific antigens using mouse-produced TCRs in patients with melanoma and colorectal cancer, respectively, yielded disease remission but also substantial impairments to healthy tissue[104,157]. Complete regression was reported with NY-ESO-1-selected $CD4^+$ ACT in a patient with metastatic melanoma[158]. TCR-T utilizing affinity-enhanced TCRs against HLA-I-restricted NY-ESO-1 and MAGE-4 cancer germline antigens resulted in confirmed partial responses in ~50% of patients with an assortment of solid tumors, mainly consisting of synovial sarcoma[109,159,160]. In multiple myeloma, the same NY-ESO-1 TCR resulted in 70% complete or near-complete responses; however, most responders relapsed within months due to immune escape[161,162]. Repurposing of a naturally occurring high-affinity HLA-II restricted regulatory T cell-derived anti-MAGE-3/6 TCR conveyed objective response in 4 of 17 patients, including one complete response in a patient with cervical carcinoma[163]. Although manageable toxicity profiles were observed for the above-mentioned trials with cancer germline antigens, TCR enhancement and repurposing bear non-negligible risks

Table 2 | Published clinical trials utilizing neoantigens as targets

Year of publication	Investigator /sponsor	Clinicaltrials.gov identifier, phase	Indication	Platform /treatment	Key results	Ref.
2014	NIH	NCT01174121, 1	Metastatic cholangiocarcinoma	Adoptive transfer of neoantigen-specific (ERBB2IP E805G) $CD4^+$ T cells isolated from tumor	Decrease in target lesions with stabilization of disease, reinjection led to tumor regression, single patient report	9
2015	Washington University	NCT00683670, 1	Stage III or IV melanoma	Intravenous application of neoepitope peptide-loaded DC vaccine	$CD8^+$ T cell responses and broadened antigenic breadth as well as clonal diversity	188,189
2016	NIH	NCT01174121, 2	Metastatic colorectal cancer	Adoptive transfer of neoantigen-specific (KRAS G12D) $CD8^+$ T cells isolated from tumor	Regression of multiple lung metastases upon infusion of four different T cell clonotypes	8
2017	BioNTech	NCT02035956, 1	Stage III or IV melanoma	Intranodal application of naked mRNA vaccine encoding for multiple neoepitopes	$CD8^+$ and especially $CD4^+$ T cell responses against multiple neoantigens, significant reduction of cumulative rate of metastatic events after vaccination	173
2017	Dana-Farber Cancer Institute	NCT01970358, 1	Stage III or IV melanoma	Subcutaneous application of peptide vaccine consisting of pooled mutated epitopes	Polyfunctional $CD8^+$ and especially $CD4^+$ T cell responses with durable memory response, recognition of autologous tumor, combination with anti-PD-1 therapy beneficial for clinical outcome	172,190
2019	Immatics	NCT02149225, 1	Glioblastoma	Intradermal application of peptide vaccine consisting of shared and mutated epitopes	$CD8^+$ and $CD4^+$ T cell responses against multiple shared and mutated epitopes	191
2019	Dana-Farber Cancer Institute	NCT02287428, 1/1b	Glioblastoma	Subcutaneous application of peptide vaccine consisting of pooled mutated epitopes	Polyfunctional $CD8^+$ and $CD4^+$ T cell responses with enriched memory phenotype and augmented T cell infiltration to the tumor	192
2020	Dana-Farber Cancer Institute /Neon Therapeutics / BioNTech US	NCT02897765, 1	Advanced melanoma, NSCLC, bladder cancer	Subcutaneous application of peptide vaccine consisting of pooled mutated epitopes combined with PD-1 blockade	Durable $CD8^+$ and especially $CD4^+$ T cell responses with cytotoxic potential, observation of epitope spreading upon vaccination	193
2020	NIH/Moderna	NCT03480152, 1	Metastatic gastrointestinal cancer	Intramuscular application of LNP-formulated mRNA vaccine encoding for multiple neoepitopes	$CD8^+$ and $CD4^+$ T cell responses against multiple mutated epitopes, small patient group ($n=4$), no objective clinical response	194
2021	NCT/ University of Heidelberg	NCT02454634, 1	Newly diagnosed glioma	Subcutaneous application of a single IDH1 (R132H) peptide vaccine	Vaccine-induced $CD4^+$ T cell responses across multiple MHC alleles in over 90% of the patients	174

for both off- and on-target adverse effects, as exemplified by cardial and neurological lethal toxicities in TCR-T trials targeting MAGE-3 antigens[106,110]. Additional ACT trials targeting self-antigens both in solid cancers (α-fetoprotein, CEA, tyrosinase, HER2, PRAME and WT1) and in leukemias (WT1, PRAME and HA-1) are currently ongoing.

The above results suggest that it is essential to improve current therapies targeting self-antigens. The success and subsequent US FDA approval of the gp100-targeting product tebentafusp is one key example. A 69% response rate was reported for DMF5 TCR-T with concurrent anti-MART-1 DC vaccination[164]. Accumulating data suggest that, despite many disappointing clinical trials of other vaccines, self-antigens can be used for vaccination when potent delivery systems of high antigen loads and proper inflammatory stimuli are used, such as in the case of mRNA vaccines (Box 2). In a phase 1 clinical trial involving 119 patients with melanoma, inoculation with self-antigen-encoding intravenously administered liposomal RNA (RNA-LPX) vaccines and anti-PD-1 therapy elicited durable objective responses in checkpoint-inhibitor-treated patients with unresectable melanoma[165]. RNA-LPX vaccines encoding the cancer testis antigens NY-ESO-1 and TPTE (transmembrane phosphatase with tension homology), the melanoma tissue-specific antigen tyrosinase and the tumor-specific antigen MAGE-A3, induced strong $CD4^+$ and $CD8^+$ T cell responses in the majority of patients, reaching low-double-digit percentages of circulating $CD8^+$ T cells. The perceived inefficiency of many therapeutic cancer vaccines may be explained, at least in part, by the requirement to not only induce potent antigen-specific T cell responses (as in the prophylactic setting), but

Table 3 | Published clinical trials utilizing viral antigens as targets with a focus on HPV

Year of publication	Investigator /sponsor	Clinicaltrials.gov identifier, phase	Indication	Platform /treatment	Key results	Ref.
2014	NA	NA, NA	Cervical intraepithelial neoplasia grade 3	Live attenuated *Lactobacillus casei* expressing full-length HPV16 E7 protein	70% of patients pathological downgrade to CIN2 at week 9 of the treatment	179
2015	National Cancer Institute	NCT01585428, 1	Metastatic HPV-associated cervical cancer	Adoptive transfer of TILs with E6 and E7 specificity	2 out of 9 patients experienced durable tumor regression	185
2016	John Hopkins University	NCT00988559, 1	Cervical intraepithelial neoplasia grade 2/3	DNA vaccine encoding for HPV antigen E7	30% of patients experienced histopathological regression	182
2018	Baylor College of Medicine	NCT02002182, 2	HPV-associated cervical cancer	Live attenuated *Listeria monocytogenes* engineered to secrete HPV proteins	Vaccine and cisplatin combination achieved 17.1% overall response rate and 38.9% overall survival at 12 months	178
2019	Isa Pharmaceuticals	NCT02426892, 2	HPV16$^+$ cancer types	Synthetic long peptide encoding for HPV antigens E6/E7	Overall response rate of 33% in combination with anti-PD-1	181
2019	National Cancer Institute	NCT02280811, 1/2	HPV16+ cancer types	Adoptive transfer of E6-specific TCR-engineered T cells	2 out of 12 patients experienced objective tumor responses	120
2020	Isa Pharmaceuticals	NCT02128126, 1/2	HPV16+ advanced, metastatic or recurrent cervical cancer	Synthetic long peptide encoding for HPV antigens E6/E7	Tumor regression in 43% of patients	182
2020	Genexine	NCT02139267, 2	Cervical intraepithelial neoplasia grade 3	DNA vaccine encoding for HPV antigens E6/E7	52% of patients experienced histopathological regression, 73% of which cleared HPV	183
2021	NA	NA, 2	Cervical intraepithelial neoplasia grade 2	Live attenuated *Lactobacillus casei* expressing full-length HPV16 E7 protein	Complete remission in 11% of the patients	180
2021	National Cancer Institute	NCT02858310, 1	HPV-associated epithelial cancers	Adoptive transfer of E7-specific TCR-engineered T cells	50% of patients experienced tumor regression with objective clinical response	119

also to drive their efficient migration into the tumor and counteract various suppressive mechanisms that tumors impose.

Clinical experience with mutation-derived neoantigens

A number of studies have reported the expansion of neoantigen-specific T cell populations following immunotherapy. ACT case studies in melanoma and head and neck cancer uncovered 8–750-fold increases in the frequency and long-term persistence of mutation-reactive clones in responders' peripheral blood[166,167]. A fivefold increase in peripheral blood neoantigen-specific T cell reactivity was detected following anti-CTLA-4 therapy in a patient with metastatic melanoma[168]. Similarly, in a case study of metastatic melanoma, a patient exhibiting a durable clinical response to combination immunotherapy consisting of an anti-PD-1 + interleukin-2-pathway agonist, was found to have tumors enriched with CD8$^+$ and CD4$^+$ neoantigen-specific T cell clones before treatment[169]. The neoantigen-reactive, but not self-antigen-specific, clones transiently expanded in the blood and modestly also within the tumor during treatment. In the case of a patient with NSCLC who responded to anti-PD-1 treatment, a neoantigen-specific T cell response could be detected in peripheral blood only after treatment initiation and increased eightfold during treatment[30]. A rise in peripheral blood neoantigen-specific T cell fractions was also noted in a patient with metastatic breast cancer, achieving complete durable regression following combination treatment with ACT, ICB and interleukin-2 (ref. 170).

Several phase 1 and 2 clinical trials that specifically target nsSNVs for therapeutic vaccination (reviewed previously[171] and summarized in Table 2) have reported promising initial results[172,173]. In one such phase 1 clinical trial, 13 patients with melanoma were vaccinated with two mRNAs, each encoding five nsSNVs. These nsSNVs were predicted from whole-exome and RNA-sequencing data of healthy and tumor tissue. For neoepitope prioritization, MHC binding affinity, expression levels and frequency of the mutated allele were considered. T cell responses against multiple neoantigens and a reduction in the cumulative rate of metastatic events were observed in all patients[173]. Of the five patients with detectable lesions, two had objective responses, one a mixed response, one a stable lymph node metastasis that was resected and one a complete response when treated in combination with anti-PD-1 blockade. The other eight patients remained tumor-free over the whole follow-up period of 12–23 months. Vaccine-induced T cell responses were observed against 60% of 125 predicted neoepitopes, of which 68% were de novo and 32% were pre-existing.

In another personalized vaccine trial, patients were vaccinated with long-peptide vaccines (15–30 amino acids) and a poly-ICLC encoding up to 20 mutations per patient[172]. Of the six patients with melanoma, four had no recurrence at 25 months. The two patients who experienced recurrence went on to complete tumor regression when treated with anti-PD-1. Vaccines induced 60% CD4$^+$ and 16% CD8$^+$ T cell responses against the 96 predicted neoantigens, which were selected from whole-exome and RNA-sequencing data using NetMHCpan to assess MHC-I binding. Mutations in oncogenes were given the highest priority during epitope selection.

A small number of reports found that infusion products highly enriched for neoantigen reactivity induced significant disease regression. For example, in a case study of cholangiocarcinoma, treatment with a TIL product consisting of 25% CD4$^+$ cells directed at an ERBB2IP^{E805G}-derived neoantigen brought about initial disease stabilization. Re-infusion of a >95% neoantigen-specific preparation upon disease progression achieved tumor regression[9]. TIL products consisting of

BOX 2

mRNA vaccines

mRNA vaccines were moved in the spotlight after the approval of prophylactic mRNA vaccines against COVID-19 during the pandemic era, although basic and translational research has shown the promise of therapeutic mRNA vaccines against various diseases, including cancer in the last three decades[165,194,213]. Currently, multiple phase 1 and 2 clinical trials against various cancer types are being conducted to assess the potential of therapeutic mRNA vaccines with early signs of clinically relevant responses.

The elegant formulation of mRNA vaccines, an otherwise fragile and short-lived intermediate for intracellular antigen production, allows its efficient transfer to APCs through various routes of administration, which in turn help induction of high and durable antigen-specific cellular and humoral immune responses. mRNA vaccines combine various features of a desirable vaccine platform. In vitro-transcribed mRNA can deliver molecularly defined antigens to be presented in the MHC-I and MHC-II context in an HLA-independent manner through expression of the whole antigen rather than selected HLA-restricted epitopes. Moreover, its intrinsic adjuvant activity obviates the need for an additional adjuvant. It also serves an ideal safety profile with immunopharmacologically optimized transient expression, the potential for repeated application, lack of genomic integration and anti-vector immunity. mRNA production scale can also be tuned easily and rapidly at a good manufacturing practice level to provide the small doses required for a single patient, as in the case of personalized cancer vaccines, or very large volumes as in the billion vaccine doses needed for global vaccination against COVID-19.

23% and 35% neoantigen-reactive cells induced prolonged, complete regressions in breast and cervical cancer cases, respectively[166,170].

Concerning recurrent neoantigens, a phase 1 IDH1-specific peptide vaccine trial, involving 33 newly diagnosed patients with grade III–IV astrocytomas resulted in vaccine-induced immune responses in 93.3% of patients[174]. Within this group of responders, a 2-year progression-free rate of 0.82 was observed. A patient with colorectal cancer treated with a TIL product highly selected (75%) for reactivity against a recurrent $KRAS^{G12D}$/HLA-C*08:02 neoantigen exhibited objective regression of multiple metastases[8]. One of the lesions progressed 9 months after therapy and has been shown to have lost the HLA-allele presenting the neopeptide. In a case report of metastatic pancreatic cancer, TCR-T against the same neoantigen resulted in objective partial response of 72% in visceral metastases, which was ongoing at 6 months[63]. In a cohort of 12 patients with chemorefractory epithelial cancers, non-selected ACT treatment with p53-reactive TIL products resulted in only two partial responses. The infused TIL products contained low frequencies of p53-reactive cells, exhibiting an exhausted phenotype[175]. Anti-p53 TCR-T of a patient with chemorefractory breast cancer resulted in objective tumor regression that lasted 6 months[175]. Unlike with self-antigens, no adverse off-target effects were reported in these highly antigen-specific ACT treatments. These studies provide direct proof of the clinical antitumor potency of mutation-derived neoantigens.

Clinical experience with viral antigens

HPV is etiologically implicated in the development of cervical and other anogenital cancers and is highly associated with oropharyngeal cancers. Prophylactic vaccines against high-risk HPV types utilize L1 bearing viral-like particles to induce neutralizing antibodies. These vaccines are estimated to prevent 90% of viral contraction and disease; however, they are ineffective in the treatment of established infections, premalignant and malignant lesions[176]. In the therapeutic setting (Table 3), the aim is to induce a cellular response in the form of HPV-specific CTLs and helper T cells, by presentation of HPV-derived antigens on APCs. The main oncogenic HPV proteins, E6 and E7, are usually targeted. Targeting of E2 may be beneficial for precancerous lesions[177]. Live vector-based vaccines, peptide/protein-based vaccines, nucleic acid-based and whole-cell vaccines have all been tried in the context of HPV (Table 3 and reviewed previously[176]). These vaccines are generally safe, well tolerated and are successful in inducing anti-HPV cellular responses; however, the observed therapeutic effects are variable and oftentimes modest compared to mouse models.

ADXS11-001, a live, attenuated *Listeria monocytogenes*-based vaccine, was evaluated in a phase 2 clinical trial in patients with relapsed/refractory cervical cancer previously treated with chemotherapy and/or radiotherapy. Combination therapy of ADXS11-001 and cisplatin achieved a 17.1% overall response rate and 38.9% overall survival at 12 months[178]. GLBL101c, a *Lactobacillus casei*-based vaccine induced regression of cervical intraepithelial neoplasia 3 (CIN3, a precancerous lesion) to CIN2 in 70% of the patients after 9 weeks of treatment[179]. In CIN2 patients the vaccine induced a response in 22%, with complete remission achieved in 11% (2 out of 19) of patients[180]. A phase 2 peptide vaccine trial queried whether combination therapy may improve upon ICB alone in patients with $HPV16^{+}$ cancer. An overall response rate of 33% was achieved for combination therapy, compared to 16–22% with anti-PD-1 alone[181]. The same vaccine achieved a tumor regression in 43% of patients with advanced, recurrent or metastatic cervical cancer in a clinical trial combining vaccination and standard chemotherapy[182]. A clinical trial with DNA vaccine GX-188e in 64 CIN3 patients induced 52% histopathological regression to ≤CIN1 at week 20 after treatment (67% at week 36). Overall, 73% of patients with proved histological regression showed HPV clearance at week 20 (77% at week 36)[183]. In a separate CIN2-3 DNA vaccine trial (pNGVL4a-CRT/E7), 30% (8 of 27) had histological regression[184].

ACT with HPV-reactive TIL products induced durable tumor regressions in two of nine patients with HPV-positive metastatic cervical cancer[185]; however, post hoc dissection revealed that other types of tumor antigens dominated these infusion products[166]. Anti-E7 TCR-T therapy led to robust tumor regression in 6 of 12 patients, whereas anti-E6 TCR-T induced responses in 2 out of 12 patients[119,120]. The experience with targeting other cancer-related viruses is currently limited but encouraging clinical responses have been observed[122,186]. Additional vaccine and ACT trials targeting cancer-related viruses, such as HPV, Epstein–Barr virus, MCPyV and HBV are currently ongoing.

Outlook

Recent years have witnessed great advances in charting tumor-antigenic landscapes, including large-scale studies of genetically encoded neoantigens and the identification of new nonself-antigen classes stemming from aberrant transcription or translation. Although the therapeutic merit of cancer-associated T cell antigens is well established, the intrinsic and contextual qualities defining effective tumor-rejection antigens are not yet fully understood. It has been argued that nonself-antigens are superior to self-antigens[151] and that clonal antigens are better than sub-clonal ones[150]; however, even though tumor antigens are usually discussed as discrete entities, the antigenic landscape within which they appear is bound to shape the immune response that they elicit[152]. For example, immunodominance may limit reactivity toward coexpressed antigens. Along with clonal fraction[150], cell-surface density may also attenuate antigenic potency and is influenced by peptide competition for HLA presentation. Processes affecting the peptide pool to increase competition may therefore drive immune evasion. The link between specific antigen traits and prototypic T cell states observed in tumors, such as exhaustion and stem-like phenotypes, also requires further elucidation. As with clonal heterogeneity[152],

a richer antigen repertoire may not automatically imply more effective antitumor immunity. New classes of cancer antigens will undoubtedly be revealed in the future, illuminating the peptidome 'dark matter' piece by piece. Translationally, such discoveries are expected to provide new targets for immunotherapy, with further delineation of the cancer HLA peptidome advancing our understanding of antitumor immunity. Moreover, it is increasingly recognized that antigen identity alone cannot predict tumor-targeting potential. Factors such as copy number and clonal fraction, as well as the identities of other antigens with which an antigen is presented (some of which may be immunodominant), may induce tolerance and substantially alter the observed immunogenicity[148,150,152,187]. A deeper understanding of the cancer immunopeptidome and dissection of the antigens at play during natural and treatment-induced antitumor immune responses will be crucial for improving immunotherapy.

References

1. Leko, V. & Rosenberg, S. A. Identifying and targeting human tumor antigens for T cell-based immunotherapy of solid tumors. *Cancer Cell* **38**, 454–472 (2020).
2. Waldman, A. D., Fritz, J. M. & Lenardo, M. J. A guide to cancer immunotherapy: from T cell basic science to clinical practice. *Nat. Rev. Immunol.* **20**, 651–668 (2020).
3. Lythe, G., Callard, R. E., Hoare, R. L. & Molina-Paris, C. How many TCR clonotypes does a body maintain. *J. Theor. Biol.* **389**, 214–224 (2016).
4. Wosen, J. E., Mukhopadhyay, D., Macaubas, C. & Mellins, E. D. Epithelial MHC class II expression and its role in antigen presentation in the gastrointestinal and respiratory tracts. *Front. Immunol.* **9**, 2144 (2018).
5. Spranger, S., Dai, D., Horton, B. & Gajewski, T. F. Tumor-residing Batf3 dendritic cells are required for effector T cell trafficking and adoptive T cell therapy. *Cancer Cell* **31**, 711–723 (2017).
6. Salmon, H. et al. Expansion and activation of CD103$^+$ dendritic cell progenitors at the tumor site enhances tumor responses to therapeutic PD-L1 and BRAF inhibition. *Immunity* **44**, 924–938 (2016).
7. Coulie, P. G., Van den Eynde, B. J., van der Bruggen, P. & Boon, T. Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. *Nat. Rev. Cancer* **14**, 135–146 (2014).
8. Tran, E. et al. T-cell transfer therapy targeting mutant KRAS in cancer. *N. Engl. J. Med.* **375**, 2255–2262 (2016).
9. Tran, E. et al. Cancer immunotherapy based on mutation-specific CD4$^+$ T cells in a patient with epithelial cancer. *Science* **344**, 641–645 (2014).
10. Garcia-Garijo, A., Fajardo, C. A. & Gros, A. Determinants for neoantigen identification. *Front. Immunol.* **10**, 1392 (2019).
11. Schumacher, T. N., Scheper, W. & Kvistborg, P. Cancer neoantigens. *Annu. Rev. Immunol.* **37**, 173–200 (2019).
12. Bartok, O. et al. Anti-tumour immunity induces aberrant peptide presentation in melanoma. *Nature* **590**, 332–337 (2021).
13. Kalaora, S. et al. Identification of bacteria-derived HLA-bound peptides in melanoma. *Nature* **592**, 138–143 (2021).
14. Ouspenskaia, T. et al. Unannotated proteins expand the MHC-I-restricted immunopeptidome in cancer. *Nat. Biotechnol.* **40**, 209–217 (2022).
15. Pearlman, A. H. et al. Targeting public neoantigens for cancer immunotherapy. *Nat. Cancer* **2**, 487–497 (2021).
16. Bakker, A. B. et al. Identification of a novel peptide derived from the melanocyte-specific gp100 antigen as the dominant epitope recognized by an HLA-A2.1-restricted anti-melanoma CTL line. *Int. J. Cancer* **62**, 97–102 (1995).
17. Brichard, V. et al. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J. Exp. Med.* **178**, 489–495 (1993).
18. Kawakami, Y. et al. Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes. *J. Exp. Med.* **180**, 347–352 (1994).
19. Nathan, P. et al. Overall survival benefit with tebentafusp in metastatic uveal melanoma. *N. Engl. J. Med.* **385**, 1196–1206 (2021).
20. Hofmann, O. et al. Genome-wide analysis of cancer/testis gene expression. *Proc. Natl Acad. Sci. USA* **105**, 20422–20427 (2008).
21. De Plaen, E. et al. Structure, chromosomal localization, and expression of 12 genes of the MAGE family. *Immunogenetics* **40**, 360–369 (1994).
22. Lurquin, C. et al. Two members of the human MAGEB gene family located in Xp21.3 are expressed in tumors of various histological origins. *Genomics* **46**, 397–408 (1997).
23. van der Bruggen, P. et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* **254**, 1643–1647 (1991).
24. Huijbers, I. J. et al. Minimal tolerance to a tumor antigen encoded by a cancer-germline gene. *J. Immunol.* **188**, 111–121 (2012).
25. Woloszynska-Read, A., Mhawech-Fauceglia, P., Yu, J., Odunsi, K. & Karpf, A. R. Intertumor and intratumor NY-ESO-1 expression heterogeneity is associated with promoter-specific and global DNA methylation status in ovarian cancer. *Clin. Cancer Res.* **14**, 3283–3290 (2008).
26. Vogelstein, B. et al. Cancer genome landscapes. *Science* **339**, 1546–1558 (2013).
27. Peri, A. et al. Combined presentation and immunogenicity analysis reveals a recurrent RAS.Q61K neoantigen in melanoma. *J. Clin. Invest.* https://doi.org/10.1172/JCI129466 (2021).
28. Duan, F. et al. Genomic and bioinformatic profiling of mutational neoepitopes reveals new rules to predict anticancer immunogenicity. *J. Exp. Med.* **211**, 2231–2248 (2014).
29. Spierings, E. et al. The minor histocompatibility antigen HA-3 arises from differential proteasome-mediated cleavage of the lymphoid blast crisis (Lbc) oncoprotein. *Blood.* **102**, 621–629 (2003).
30. Rizvi, N. A. et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348**, 124–128 (2015).
31. Snyder, A. et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N. Engl. J. Med.* **371**, 2189–2199 (2014).
32. Van Allen, E. M. et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* **350**, 207–211 (2015).
33. Lauss, M. et al. Mutational and putative neoantigen load predict clinical benefit of adoptive T cell therapy in melanoma. *Nat. Commun.* **8**, 1738 (2017).
34. Overman, M. J. et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol.* **18**, 1182–1191 (2017).
35. Le, D. T. et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N. Engl. J. Med.* **372**, 2509–2520 (2015).
36. Le, D. T. et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* **357**, 409–413 (2017).
37. Yarchoan, M., Hopkins, A. & Jaffee, E. M. Tumor mutational burden and response rate to PD-1 inhibition. *N. Engl. J. Med.* **377**, 2500–2501 (2017).
38. Cercek, A. et al. PD-1 blockade in mismatch repair-deficient, locally advanced rectal cancer. *N. Engl. J. Med.* **386**, 2363–2376 (2022).
39. Merchant, M. et al. Tumor mutational burden and immunotherapy in gliomas. *Trends Cancer* **7**, 1054–1058 (2021).
40. Segal, N. H. et al. Epitope landscape in breast and colorectal cancer. *Cancer Res.* **68**, 889–892 (2008).
41. Kalaora, S. & Samuels, Y. in *Cancer Immunosurveillance* (eds. López-Soto, A. & Folgueras, A. R.) 203–214 (Springer, 2019).

42. Bassani-Sternberg, M. & Coukos, G. Mass spectrometry-based antigen discovery for cancer immunotherapy. *Curr. Opin. Immunol.* **41**, 9–17 (2016).
43. Castle, J. C. et al. Exploiting the mutanome for tumor vaccination. *Cancer Res.* **72**, 1081–1091 (2012).
44. Clark, R. E. et al. Direct evidence that leukemic cells present HLA-associated immunogenic peptides derived from the BCR-ABL b3a2 fusion protein. *Blood* **98**, 2887–2893 (2001).
45. van der Lee, D. I. et al. Mutated nucleophosmin 1 as immunotherapy target in acute myeloid leukemia. *J. Clin. Invest.* **129**, 774–785 (2019).
46. McGranahan, N. et al. Clonal status of actionable driver events and the timing of mutational processes in cancer evolution. *Sci. Transl. Med.* **7**, 283ra254 (2015).
47. Wolfel, T. et al. A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science* **269**, 1281–1284 (1995).
48. Robbins, P. F. et al. A mutated β-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. *J. Exp. Med.* **183**, 1185–1192 (1996).
49. Kvistborg, P. et al. TIL therapy broadens the tumor-reactive $CD8^+$ T cell compartment in melanoma patients. *Oncoimmunology* **1**, 409–418 (2012).
50. Leisegang, M., Kammertoens, T., Uckert, W. & Blankenstein, T. Targeting human melanoma neoantigens by T cell receptor gene therapy. *J. Clin. Invest.* **126**, 854–858 (2016).
51. Gjertsen, M. K., Saeterdal, I., Saeboe-Larssen, S. & Gaudernack, G. HLA-A3 restricted mutant ras specific cytotoxic T-lymphocytes induced by vaccination with T-helper epitopes. *J. Mol. Med.* **81**, 43–50 (2003).
52. Gjertsen, M. K., Bjorheim, J., Saeterdal, I., Myklebust, J. & Gaudernack, G. Cytotoxic $CD4^+$ and $CD8^+$ T lymphocytes, generated by mutant p21-ras (12Val) peptide vaccination of a patient, recognize 12Val-dependent nested epitopes present within the vaccine peptide and kill autologous tumour cells carrying this mutation. *Int. J. Cancer* **72**, 784–790 (1997).
53. Abrams, S. I. et al. Generation of stable $CD4^+$ and $CD8^+$ T cell lines from patients immunized with ras oncogene-derived peptides reflecting codon 12 mutations. *Cell Immunol.* **182**, 137–151 (1997).
54. Ito, D. 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* **120**, 2618–2624 (2007).
55. Malekzadeh, P. et al. Antigen experienced T cells from peripheral blood recognize p53 neoantigens. *Clin. Cancer Res.* **26**, 1267–1276 (2020).
56. Sharkey, M. S., Lizee, G., Gonzales, M. I., Patel, S. & Topalian, S. L. $CD4^+$ T-cell recognition of mutated B-RAF in melanoma patients harboring the V599E mutation. *Cancer Res.* **64**, 1595–1599 (2004).
57. Somasundaram, R. et al. Human leukocyte antigen-A2-restricted CTL responses to mutated BRAF peptides in melanoma patients. *Cancer Res.* **66**, 3287–3293 (2006).
58. Andersen, M. H. et al. Immunogenicity of constitutively active V599EBRaf. *Cancer Res.* **64**, 5456–5460 (2004).
59. Yamada, T. et al. EGFR T790M mutation as a possible target for immunotherapy; identification of HLA-A*0201-restricted T cell epitopes derived from the EGFR T790M mutation. *PLoS ONE* **8**, e78389 (2013).
60. Chandran, S. S. et al. Immunogenicity and therapeutic targeting of a public neoantigen derived from mutated PIK3CA. *Nat. Med.* https://doi.org/10.1038/s41591-022-01786-3 (2022).
61. Kalaora, S. et al. Combined analysis of antigen presentation and T-cell recognition reveals restricted immune responses in melanoma. *Cancer Discov.* **8**, 1366–1375 (2018).
62. Wang, Q. et al. Direct detection and quantification of neoantigens. *Cancer Immunol. Res.* **7**, 1748–1754 (2019).
63. Leidner, R. et al. Neoantigen T-cell receptor gene therapy in pancreatic cancer. *N. Engl. J. Med.* **386**, 2112–2119 (2022).
64. Turajlic, S. et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: a pan-cancer analysis. *Lancet Oncol.* **18**, 1009–1021 (2017).
65. Mansfield, A. S. et al. Neoantigenic potential of complex chromosomal rearrangements in mesothelioma. *J. Thorac. Oncol.* **14**, 276–287 (2019).
66. Quintas-Cardama, A. & Cortes, J. Molecular biology of bcr-abl1-positive chronic myeloid leukemia. *Blood* **113**, 1619–1630 (2009).
67. Sasaki, T., Rodig, S. J., Chirieac, L. R. & Janne, P. A. The biology and treatment of EML4-ALK non-small cell lung cancer. *Eur. J. Cancer* **46**, 1773–1780 (2010).
68. Wei, Z. et al. The landscape of tumor fusion neoantigens: a pan-cancer analysis. *iScience* **21**, 249–260 (2019).
69. Yang, W. et al. Immunogenic neoantigens derived from gene fusions stimulate T cell responses. *Nat. Med.* **25**, 767–775 (2019).
70. Chaisson, M. J. P. et al. Multi-platform discovery of haplotype-resolved structural variation in human genomes. *Nat. Commun.* **10**, 1784 (2019).
71. Sethi, R. et al. Integrative analysis of structural variations using short-reads and linked-reads yields highly specific and sensitive predictions. *PLoS Comput. Biol.* **16**, e1008397 (2020).
72. Hu, X. et al. TumorFusions: an integrative resource for cancer-associated transcript fusions. *Nucleic Acids Res.* **46**, D1144–D1149 (2018).
73. Jurtz, V. et al. NetMHCpan-4.0: improved peptide-MHC class I interaction predictions integrating eluted ligand and peptide binding affinity data. *J. Immunol.* **199**, 3360–3368 (2017).
74. Wang, S., Mao, C. & Liu, S. Peptides encoded by noncoding genes: challenges and perspectives. *Signal Transduct. Target Ther.* **4**, 57 (2019).
75. Guilloux, Y. et al. A peptide recognized by human cytolytic T lymphocytes on HLA-A2 melanomas is encoded by an intron sequence of the *N*-acetylglucosaminyltransferase V gene. *J. Exp. Med.* **183**, 1173–1183 (1996).
76. Lupetti, R. et al. Translation of a retained intron in tyrosinase-related protein (TRP) 2 mRNA generates a new cytotoxic T lymphocyte (CTL)-defined and shared human melanoma antigen not expressed in normal cells of the melanocytic lineage. *J. Exp. Med.* **188**, 1005–1016 (1998).
77. Chong, C. et al. Integrated proteogenomic deep sequencing and analytics accurately identify non-canonical peptides in tumor immunopeptidomes. *Nat. Commun.* **11**, 1293 (2020).
78. Robbins, P. F. et al. The intronic region of an incompletely spliced gp100 gene transcript encodes an epitope recognized by melanoma-reactive tumor-infiltrating lymphocytes. *J. Immunol.* **159**, 303–308 (1997).
79. Uenaka, A. et al. Identification of a unique antigen peptide pRL1 on BALB/c RL male 1 leukemia recognized by cytotoxic T lymphocytes and its relation to the Akt oncogene. *J. Exp. Med.* **180**, 1599–1607 (1994).
80. Wang, R. F. et al. A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. *J. Immunol.* **161**, 3598–3606 (1998).
81. Graddis, T. J. et al. Tumor immunotherapy with alternative reading frame peptide antigens. *Immunobiology* **209**, 535–544 (2004).
82. Weinzierl, A. O. et al. A cryptic vascular endothelial growth factor T-cell epitope: identification and characterization by mass spectrometry and T-cell assays. *Cancer Res.* **68**, 2447–2454 (2008).

83. Ronsin, C. et al. A non-AUG-defined alternative open reading frame of the intestinal carboxyl esterase mRNA generates an epitope recognized by renal cell carcinoma-reactive tumor-infiltrating lymphocytes in situ. *J. Immunol.* **163**, 483–490 (1999).
84. Ho, O. & Green, W. R. Alternative translational products and cryptic T cell epitopes: expecting the unexpected. *J. Immunol.* **177**, 8283–8289 (2006).
85. Lee, J. Y. et al. Promiscuous methionyl-tRNA synthetase mediates adaptive mistranslation to protect cells against oxidative stress. *J. Cell Sci.* **127**, 4234–4245 (2014).
86. Netzer, N. et al. Innate immune and chemically triggered oxidative stress modifies translational fidelity. *Nature* **462**, 522–526 (2009).
87. Santos, M. et al. Codon misreading tRNAs promote tumor growth in mice. *RNA Biol.* **15**, 773–786 (2018).
88. Chen, L., Liu, S. & Tao, Y. Regulating tumor suppressor genes: post-translational modifications. *Signal Transduct. Target Ther.* **5**, 90 (2020).
89. Kacen, A. et al. Post-translational modifications reshape the antigenic landscape of the MHC I immunopeptidome in tumors. *Nat. Biotechnol.* **41**, 239–251 (2023).
90. zur Hausen, H. Viruses in human cancers. *Science* **254**, 1167–1173 (1991).
91. Wroblewski, L. E., Peek, R. M. Jr. & Wilson, K. T. *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. *Clin. Microbiol. Rev.* **23**, 713–739 (2010).
92. Lin, L. et al. The burden and trends of primary liver cancer caused by specific etiologies from 1990 to 2017 at the global, regional, national, age, and sex level results from the Global Burden of Disease study 2017. *Liver Cancer* **9**, 563–582 (2020).
93. Bhatt, K. H. et al. Profiling HPV-16-specific T cell responses reveals broad antigen reactivities in oropharyngeal cancer patients. *J. Exp. Med.* https://doi.org/10.1084/jem.20200389 (2020).
94. Chabeda, A. et al. Therapeutic vaccines for high-risk HPV-associated diseases. *Papillomavirus Res.* **5**, 46–58 (2018).
95. Quiding-Jarbrink, M., Lundin, B. S., Lonroth, H. & Svennerholm, A. M. $CD4^+$ and $CD8^+$ T cell responses in Helicobacter pylori-infected individuals. *Clin. Exp. Immunol.* **123**, 81–87 (2001).
96. van Zyl, D. G., Mautner, J. & Delecluse, H. J. Progress in EBV vaccines. *Front. Oncol.* **9**, 104 (2019).
97. Fluckiger, A. et al. Cross-reactivity between tumor MHC class I-restricted antigens and an enterococcal bacteriophage. *Science* **369**, 936–942 (2020).
98. Ficial, M. et al. Expression of T-cell exhaustion molecules and human endogenous retroviruses as predictive biomarkers for response to nivolumab in metastatic clear cell renal cell carcinoma. *Clin. Cancer Res.* **27**, 1371–1380 (2021).
99. Jin, B. Y. et al. Engineered T cells targeting E7 mediate regression of human papillomavirus cancers in a murine model. *JCI Insight* https://doi.org/10.1172/jci.insight.99488 (2018).
100. Draper, L. M. et al. Targeting of HPV-16^+ epithelial cancer cells by TCR gene engineered T Cells directed against E6. *Clin. Cancer Res.* **21**, 4431–4439 (2015).
101. Narunsky-Haziza, L. et al. Pan-cancer analyses reveal cancer-type-specific fungal ecologies and bacteriome interactions. *Cell* **185**, 3789–3806 e3717 (2022).
102. Caballero, O. L. & Chen, Y. T. Cancer/testis (CT) antigens: potential targets for immunotherapy. *Cancer Sci.* **100**, 2014–2021 (2009).
103. Nejman, D. et al. The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science* **368**, 973–980 (2020).
104. Johnson, L. A. et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood* **114**, 535–546 (2009).
105. van den Berg, J. H. et al. Case report of a fatal serious adverse event upon administration of T cells transduced with a MART-1-specific T-cell receptor. *Mol. Ther.* **23**, 1541–1550 (2015).
106. Linette, G. P. et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood* **122**, 863–871 (2013).
107. Morgan, R. A. et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science* **314**, 126–129 (2006).
108. Nowicki, T. S. et al. A pilot trial of the combination of transgenic NY-ESO-1-reactive adoptive cellular therapy with dendritic cell vaccination with or without ipilimumab. *Clin. Cancer Res.* **25**, 2096–2108 (2019).
109. D'Angelo, S. P. et al. Antitumor activity associated with prolonged persistence of adoptively transferred NY-ESO-1 (c259)T cells in synovial sarcoma. *Cancer Discov.* **8**, 944–957 (2018).
110. Morgan, R. A. et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J. Immunother.* **36**, 133–151 (2013).
111. Fritsch, E. F. et al. HLA-binding properties of tumor neoepitopes in humans. *Cancer Immunol. Res.* **2**, 522–529 (2014).
112. Sim, M. J. W. et al. High-affinity oligoclonal TCRs define effective adoptive T cell therapy targeting mutant KRAS-G12D. *Proc. Natl Acad. Sci. USA* **117**, 12826–12835 (2020).
113. Martincorena, I. et al. Somatic mutant clones colonize the human esophagus with age. *Science* **362**, 911–917 (2018).
114. Martincorena, I. et al. Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science* **348**, 880–886 (2015).
115. Cuevas, M. V. R. et al. Most non-canonical proteins uniquely populate the proteome or immunopeptidome. *Cell Rep.* **34**, 108815 (2021).
116. Pataskar, A. et al. Tryptophan depletion results in tryptophan-to-phenylalanine substitutants. *Nature* **603**, 721–727 (2022).
117. Liu, W. et al. Identifying the target cells and mechanisms of merkel cell polyomavirus infection. *Cell Host Microbe* **19**, 775–787 (2016).
118. Tan, A. T. & Schreiber, S. Adoptive T-cell therapy for HBV-associated HCC and HBV infection. *Antiviral Res.* **176**, 104748 (2020).
119. Nagarsheth, N. B. et al. TCR-engineered T cells targeting E7 for patients with metastatic HPV-associated epithelial cancers. *Nat. Med.* **27**, 419–425 (2021).
120. Doran, S. L. et al. T-cell receptor gene therapy for human papillomavirus-associated epithelial cancers: a first-in-human, phase I/II study. *J. Clin. Oncol.* **37**, 2759–2768 (2019).
121. Kwong, D. L. W., Lee, V. H. F. & Nicholls, J. M. in *Nasopharyngeal Carcinoma* (eds Anne W. M. Lee, Maria Li Lung, & Wai Tong Ng) 337–351 (Academic Press, 2019).
122. Paulson, K. G. et al. Acquired cancer resistance to combination immunotherapy from transcriptional loss of class I HLA. *Nat. Commun.* **9**, 3868 (2018).
123. Yan, F. M. et al. Hepatitis C virus may infect extrahepatic tissues in patients with hepatitis C. *World J. Gastroenterol.* **6**, 805–811 (2000).
124. McMahan, R. H. & Slansky, J. E. Mobilizing the low-avidity T cell repertoire to kill tumors. *Semin. Cancer Biol.* **17**, 317–329 (2007).
125. Thomas, S. et al. Human T cells expressing affinity-matured TCR display accelerated responses but fail to recognize low density of MHC-peptide antigen. *Blood* **118**, 319–329 (2011).
126. Engels, B., Chervin, A. S., Sant, A. J., Kranz, D. M. & Schreiber, H. Long-term persistence of CD4(+) but rapid disappearance of CD8(+) T cells expressing an MHC class I-restricted TCR of nanomolar affinity. *Mol. Ther.* **20**, 652–660 (2012).
127. Schmid, D. A. et al. Evidence for a TCR affinity threshold delimiting maximal CD8 T cell function. *J. Immunol.* **184**, 4936–4946 (2010).

128. Galvez, J., Galvez, J. J. & Garcia-Penarrubia, P. Is TCR/pMHC affinity a good estimate of the T-cell response? An answer based on predictions from 12 phenotypic models. *Front. Immunol.* **10**, 349 (2019).
129. Oliveira, G. et al. Phenotype, specificity and avidity of antitumour CD8(+) T cells in melanoma. *Nature* **596**, 119–125 (2021).
130. Aleksic, M. et al. Different affinity windows for virus and cancer-specific T-cell receptors: implications for therapeutic strategies. *Eur. J. Immunol.* **42**, 3174–3179 (2012).
131. Hoffmann, M. M. & Slansky, J. E. T-cell receptor affinity in the age of cancer immunotherapy. *Mol. Carcinog.* **59**, 862–870 (2020).
132. Smith, S. N. et al. Changing the peptide specificity of a human T-cell receptor by directed evolution. *Nat. Commun.* **5**, 5223 (2014).
133. Bassan, D. et al. Avidity optimization of a MAGE-A1-specific TCR with somatic hypermutation. *Eur. J. Immunol.* **51**, 1505–1518 (2021).
134. Hsiue, E. H. et al. Targeting a neoantigen derived from a common TP53 mutation. *Science* https://doi.org/10.1126/science.abc8697 (2021).
135. Douglass, J. et al. Bispecific antibodies targeting mutant RAS neoantigens. *Sci. Immunol.* https://doi.org/10.1126/sciimmunol.abd5515 (2021).
136. Hwang, M. S. et al. Structural engineering of chimeric antigen receptors targeting HLA-restricted neoantigens. *Nat. Commun.* **12**, 5271 (2021).
137. Poorebrahim, M. et al. TCR-like CARs and TCR-CARs targeting neoepitopes: an emerging potential. *Cancer Gene Ther.* **28**, 581–589 (2021).
138. Poole, A. et al. Therapeutic high affinity T cell receptor targeting a KRASG12D cancer neoantigen. *Nat. Commun.* **13**, 5333 (2022).
139. Gonzalez, P. A. et al. T cell receptor binding kinetics required for T cell activation depend on the density of cognate ligand on the antigen-presenting cell. *Proc. Natl Acad. Sci. USA* **102**, 4824–4829 (2005).
140. Engels, B. et al. Relapse or eradication of cancer is predicted by peptide-major histocompatibility complex affinity. *Cancer Cell* **23**, 516–526 (2013).
141. Yu, Z. et al. Poor immunogenicity of a self/tumor antigen derives from peptide-MHC-I instability and is independent of tolerance. *J. Clin. Invest.* **114**, 551–559 (2004).
142. Purbhoo, M. A. et al. Quantifying and imaging NY-ESO-1/LAGE-1-derived epitopes on tumor cells using high affinity T cell receptors. *J. Immunol.* **176**, 7308–7316 (2006).
143. Stopfer, L. E. et al. Absolute quantification of tumor antigens using embedded MHC-I isotopologue calibrants. *Proc. Natl Acad. Sci. USA* https://doi.org/10.1073/pnas.2111173118 (2021).
144. Apavaloaei, A., Hardy, M. P., Thibault, P. & Perreault, C. The origin and immune recognition of tumor-specific antigens. *Cancers* https://doi.org/10.3390/cancers12092607 (2020).
145. Ashrafi, G. H., Haghshenas, M. R., Marchetti, B., O'Brien, P. M. & Campo, M. S. E5 protein of human papillomavirus type 16 selectively downregulates surface HLA class I. *Int. J. Cancer* **113**, 276–283 (2005).
146. Jaeger, A. M. et al. Deciphering the tumor-specific immunopeptidome in vivo with genetically engineered mouse models. *Nature* https://doi.org/10.1038/s41586-022-04839-2 (2022).
147. Norbury, C. C. et al. CD8+ T cell cross-priming via transfer of proteasome substrates. *Science* **304**, 1318–1321 (2004).
148. Westcott, P. M. K. et al. Low neoantigen expression and poor T-cell priming underlie early immune escape in colorectal cancer. *Nat. Cancer* **2**, 1071–1085 (2021).
149. Wolf, Y. & Samuels, Y. Intratumor heterogeneity and antitumor immunity shape one another bidirectionally. *Clin. Cancer Res.* **28**, 2994–3001 (2022).
150. Gejman, R. S. et al. Rejection of immunogenic tumor clones is limited by clonal fraction. *eLife* https://doi.org/10.7554/elife.41090 (2018).
151. McGranahan, N. et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* **351**, 1463–1469 (2016).
152. Wolf, Y. et al. UVB-induced tumor heterogeneity diminishes immune response in melanoma. *Cell.* **179**, 219–235 (2019).
153. Rosenthal, R. et al. Neoantigen-directed immune escape in lung cancer evolution. *Nature* **567**, 479–485 (2019).
154. Lowery, F. J. et al. Molecular signatures of antitumor neoantigen-reactive T cells from metastatic human cancers. *Science* **375**, 877–884 (2022).
155. Caushi, J. X. et al. Author Correction: Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers. *Nature* https://doi.org/10.1038/s41586-021-03893-6 (2021).
156. Caushi, J. X. et al. Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers. *Nature* **596**, 126–132 (2021).
157. Parkhurst, M. R. et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. *Mol. Ther.* **19**, 620–626 (2011).
158. Hunder, N. N. et al. Treatment of metastatic melanoma with autologous $CD4^+$ T cells against NY-ESO-1. *N. Engl. J. Med.* **358**, 2698–2703 (2008).
159. Robbins, P. F. et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J. Clin. Oncol.* **29**, 917 (2011).
160. Anon. T cells targeting MAGE-A4 shrink tumors. *Cancer Discov.* https://doi.org/10.1158/2159-8290.CD-NB2020-059 (2020).
161. Rapoport, A. P. et al. NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma. *Nat. Med.* **21**, 914–921 (2015).
162. Klippel, Z. K. et al. Immune escape from NY-ESO-1-specific T-cell therapy via loss of heterozygosity in the MHC. *Gene Ther.* **21**, 337–342 (2014).
163. Lu, Y. C. et al. Treatment of patients with metastatic cancer using a major histocompatibility complex class II-restricted T-cell receptor targeting the cancer germline antigen MAGE-A3. *J. Clin. Oncol.* **35**, 3322–3329 (2017).
164. Chodon, T. et al. Adoptive transfer of MART-1 T-cell receptor transgenic lymphocytes and dendritic cell vaccination in patients with metastatic melanoma. *Clin. Cancer Res.* **20**, 2457–2465 (2014).
165. Sahin, U. et al. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. *Nature* **585**, 107–112 (2020).
166. Stevanovic, S. et al. Landscape of immunogenic tumor antigens in successful immunotherapy of virally induced epithelial cancer. *Science* **356**, 200–205 (2017).
167. van den Berg, J. H. et al. Tumor infiltrating lymphocytes (TIL) therapy in metastatic melanoma: boosting of neoantigen-specific T cell reactivity and long-term follow-up. *J. Immunother. Cancer* https://doi.org/10.1136/jitc-2020-000848 (2020).
168. van Rooij, N. et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. *J. Clin. Oncol.* **31**, e439–e442 (2013).
169. Veatch, J. R. et al. Mobilization of pre-existing polyclonal T cells specific to neoantigens but not self-antigens during treatment of a patient with melanoma with bempegaldesleukin and nivolumab. *J. Immunother. Cancer* **8**, e001591 (2020).
170. Zacharakis, N. et al. Immune recognition of somatic mutations leading to complete durable regression in metastatic breast cancer. *Nat. Med.* **24**, 724–730 (2018).
171. Shemesh, C. S. et al. Personalized cancer vaccines: clinical landscape, challenges, and opportunities. *Mol. Ther.* **29**, 555–570 (2021).

172. Ott, P. A. et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature* **547**, 217–221 (2017).
173. Sahin, U. et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* **547**, 222–226 (2017).
174. Platten, M. et al. A vaccine targeting mutant IDH1 in newly diagnosed glioma. *Nature* **592**, 463–468 (2021).
175. Kim, S. P. et al. Adoptive cellular therapy with autologous tumor-infiltrating lymphocytes and T-cell Receptor-engineered T cells targeting common p53 neoantigens in human solid tumors. *Cancer Immunol. Res.* https://doi.org/10.1158/2326-6066.CIR-22-0040 (2022).
176. Mo, Y. et al. Prophylactic and therapeutic HPV vaccines: current scenario and perspectives. *Front. Cell Infect. Microbiol.* **12**, 909223 (2022).
177. Smith, J. A. et al. SMCX and components of the TIP60 complex contribute to E2 regulation of the HPV E6/E7 promoter. *Virology* **468-470**, 311–321 (2014).
178. Basu, P. et al. A Randomized Phase 2 Study of ADXS11-001 Listeria monocytogenes-Listeriolysin O Immunotherapy With or Without Cisplatin in Treatment of Advanced Cervical Cancer. *Int. J. Gynecol. Cancer* **28**, 764–772 (2018).
179. Kawana, K. et al. Oral vaccination against HPV E7 for treatment of cervical intraepithelial neoplasia grade 3 (CIN3) elicits E7-specific mucosal immunity in the cervix of CIN3 patients. *Vaccine* **32**, 6233–6239 (2014).
180. Ikeda, Y. et al. A placebo-controlled, double-blind randomized (phase IIB) trial of oral administration with HPV16 E7-expressing *Lactobacillus*, GLBL101c, for the treatment of cervical intraepithelial neoplasia grade 2 (CIN2). *Vaccines* https://doi.org/10.3390/vaccines9040329 (2021).
181. Massarelli, E. et al. Combining immune checkpoint blockade and tumor-specific vaccine for patients with incurable human papillomavirus 16-related cancer: a phase 2 clinical trial. *JAMA Oncol.* **5**, 67–73 (2019).
182. Melief, C. J. M. et al. Strong vaccine responses during chemotherapy are associated with prolonged cancer survival. *Sci. Transl. Med.* https://doi.org/10.1126/scitranslmed.aaz8235 (2020).
183. Choi, Y. J. et al. A phase II, prospective, randomized, multicenter, open-label study of GX-188E, an HPV DNA Vaccine, in patients with cervical intraepithelial neoplasia 3. *Clin. Cancer Res.* **26**, 1616–1623 (2020).
184. Alvarez, R. D. et al. A pilot study of pNGVL4a-CRT/E7(detox) for the treatment of patients with HPV16^{+} cervical intraepithelial neoplasia 2/3 (CIN2/3). *Gynecol. Oncol.* **140**, 245–252 (2016).
185. Stevanovic, S. et al. Complete regression of metastatic cervical cancer after treatment with human papillomavirus-targeted tumor-infiltrating T cells. *J. Clin. Oncol.* **33**, 1543–1550 (2015).
186. Chow, J. C., Ngan, R. K., Cheung, K. M. & Cho, W. C. Immunotherapeutic approaches in nasopharyngeal carcinoma. *Expert Opin. Biol. Ther.* **19**, 1165–1172 (2019).
187. Burger, M. L. et al. Antigen dominance hierarchies shape TCF1(+) progenitor CD8 T cell phenotypes in tumors. *Cell* **184**, 4996–5014 (2021).
188. Carreno, B. M. et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science* **348**, 803–808 (2015).
189. Kreiter, S. et al. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. *Nature* **520**, 692–696 (2015).
190. Hu, Z. et al. Personal neoantigen vaccines induce persistent memory T cell responses and epitope spreading in patients with melanoma. *Nat. Med.* **27**, 515–525 (2021).
191. Hilf, N. et al. Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature* **565**, 240–245 (2019).
192. Keskin, D. B. et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* **565**, 234–239 (2019).
193. Ott, P. A. et al. A phase Ib trial of personalized neoantigen therapy plus anti-PD-1 in patients with advanced melanoma, non-small cell lung cancer, or bladder cancer. *Cell* **183**, 347–362 (2020).
194. Cafri, G. et al. mRNA vaccine-induced neoantigen-specific T cell immunity in patients with gastrointestinal cancer. *J. Clin. Invest.* **130**, 5976–5988 (2020).
195. Gross, L. Intradermal immunization of C3H mice against a sarcoma that originated in an animal of the same line. *Cancer Res.* **3**, 326–333 (1943).
196. Miller, J. F. & Mitchell, G. F. The thymus and the precursors of antigen reactive cells. *Nature* **216**, 659–663 (1967).
197. Cerottini, J. C. & Brunner, K. T. Cell-mediated cytotoxicity, allograft rejection, and tumor immunity. *Adv. Immunol.* **18**, 67–132 (1974).
198. Rosenberg, S. A. et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. *N. Engl. J. Med.* **319**, 1676–1680 (1988).
199. De Plaen, E. et al. Immunogenic (tum-) variants of mouse tumor P815: cloning of the gene of tum-antigen P91A and identification of the tum-mutation. *Proc. Natl Acad. Sci. USA* **85**, 2274–2278 (1988).
200. Traversari, C. et al. A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. *J. Exp. Med.* **176**, 1453–1457 (1992).
201. Fisk, B., Blevins, T. L., Wharton, J. T. & Ioannides, C. G. Identification of an immunodominant peptide of HER-2/neu protooncogene recognized by ovarian tumor-specific cytotoxic T lymphocyte lines. *J. Exp. Med.* **181**, 2109–2117 (1995).
202. Acres, B. & Limacher, J.-M. MUC1 as a target antigen for cancer immunotherapy. *Expert Rev. Vaccines* **4**, 493–502 (2005).
203. Wood, L. D. et al. The genomic landscapes of human breast and colorectal cancers. *Science* **318**, 1108–1113 (2007).
204. Cheever, M. A. & Higano, C. S. PROVENGE (Sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. *Clin. Cancer Res.* **17**, 3520–3526 (2011).
205. Fellner, C. Ipilimumab (yervoy) prolongs survival in advanced melanoma: serious side effects and a hefty price tag may limit its use. *P T* **37**, 503–530 (2012).
206. Raedler, L. A. Keytruda (pembrolizumab): first PD-1 Inhibitor approved for previously treated unresectable or metastatic melanoma. *Am. Health Drug Benefits* **8**, 96–100 (2015).
207. Raedler, L. A. Opdivo (nivolumab): second PD-1 inhibitor receives FDA approval for unresectable or metastatic melanoma. *Am. Health Drug Benefits* **8**, 180–183 (2015).
208. Rosenberg, S. A. et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin. Cancer Res.* **17**, 4550–4557 (2011).
209. Rosenberg, S. A. & Restifo, N. P. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science* **348**, 62–68 (2015).
210. Sahin, U. & Tureci, O. Personalized vaccines for cancer immunotherapy. *Science* **359**, 1355–1360 (2018).
211. Tumeh, P. C. et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515**, 568–571 (2014).
212. Wei, S. C., Duffy, C. R. & Allison, J. P. Fundamental mechanisms of immune checkpoint blockade therapy. *Cancer Discov.* **8**, 1069–1086 (2018).
213. Polack, F. P. et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. *N. Engl. J. Med.* **383**, 2603–2615 (2020).

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