**What do cancer-specific CD8+ T cells see? - The contribution of immunopeptidomics**

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**Abstract**

Immunopeptidomics is the survey of all peptides displayed on a cell or tissue when bound to HLA molecules using tandem mass spectrometry. When attempting to determine the targets of tumour-specific CD8+ T cells, a survey of the potential ligands in tumour tissues is invaluable, and, in comparison with in-silico predictions, provides greater certainty of the existence of individual epitopes, as immunopeptidomics-confirmed CD8+ T cell epitopes are known to be immunogenic, and direct observation should avoid the risk of autoreactivity which could arise following immunisation with structural homologues. The canonical sources of CD8+ T cell tumour specific epitopes, such as tumour associated antigens, may be well conserved between patients and tumour types, but are often only weakly immunogenic. Direct observation of tumour-specific neoantigens by immunopeptidomics is rare, although valuable. Thus, there has been increasing interest in the non-canonical origins of tumour-reactive CD8+ T cell epitopes, such as those arising from proteasomal splicing events, translational/turnover defects and alternative open reading frame reads. Such epitopes can be identified in-silico, although validation is more challenging. Non-self CD8+ T cell epitopes such as viral epitopes may be useful in certain cancer types with known viral origins, however these have been relatively unexplored with immunopeptidomics to date, possibly due to the paucity of source viral proteins in tumour tissues. This review examines the latest evidence for canonical, non-canonical and non-human CD8+ T cell epitopes identified by immunopeptidomics, and concludes that the relative contribution for each of these sources to anti-tumour CD8+ T cell reactivity is currently uncertain.

**Introduction**

Cancers are recognised by every part of the immune system, including both the innate and adaptive arms. Humoral and cell-mediated recognition of tumour cells is dependent on difference from self. A major challenge posed to the immune system by cancers is that, unlike pathogens, they are derived from host cells and therefore closely resemble self in most of their molecular characteristics. Priming for the recognition of tumours by T cells requires presentation of tumour antigens on both human leukocyte antigens (HLA) class-I and -II on the surface of antigen presenting cells (APC) to stimulate both CD4+ and CD8+ T cells (1). Thus CD8+ T cell responses are initially primed by cross-presentation of tumour antigen-derived peptides principally in dendritic cells (DC). Subsequent tumour cell killing by cytotoxic CD8+ T cells requires presentation of tumour antigens on HLA-I of the tumour cells themselves (2).

Compounding the challenge of generating sufficient quality CD8+ T cell recognition of tumour cells, the tumour microenvironment (TME) is immunosuppressive (3). Tumour-infiltrating CD8+ T cells often display markers of exhaustion, in particular the immunomodulatory regulator proteins PD-1 and CTLA-4, and other exhaustion markers such as TIM-3 and LAG-3 (4). To further augment the immunosuppressive environment, tumour cells can express PD-L1, the natural ligand of PD-1, further suppressing T cell function.

In normal tissues, cellular proteins are processed and presented on HLA-I and, due to thymic deletion of self-recognising T cells, do not initiate a cytotoxic T lymphocyte (CTL) response. When a tumour is present, any tumour-specific CD8+ T cells will then have the potential to be re-activated through interaction with HLA-I presented tumour-specific peptides on the tumour cells, initiating cytotoxic activity and tumour cell elimination.

The use of immune checkpoint inhibitors (ICI) such as PD-1 blocking antibodies has revolutionised cancer therapy. Whilst they do not help every patient with every tumour type, they do demonstrate the existence of a primed tumour-specific CD8+ T cell population in the circulation and the tumour microenvironment (5). Defects in the HLA-I processing and presentation machinery in tumour cells can occur, although complete loss of HLA-I expression is unlikely due to consequent elimination by NK cells, it is thought that surface HLA-I downregulation may be an immune escape strategy (6) but it’s exact role in ICI resistance is unclear. One significant question remains as to what anti-tumour CD8+ T cells might recognise and whether this can be harnessed to create therapeutics specifically targeting them.

Peptides presented by HLA-I are derived by proteolysis of intracellular proteins and bound as part of a heterotrimeric peptide complex (pHLA-I) consisting of peptide, HLA heavy chain and a light chain (b2-microglobulin). The HLA genes are polygenic, with 6 alleles present in every individual, and are also highly polymorphic resulting in a vast potential diversity of bound peptides, as each allele contains optimal peptide binding motifs at different amino acid positions, and optimal peptide length preference, with the dominant length across all HLA-I alleles being 9 amino acids long.

Immunopeptidomics is the survey of all of the HLA-bound peptides in a cell line or tissue. It is achieved through acid elution of the HLA-bound peptides either directly from the cells/tissues or from immune-captured peptide/HLA complexes, followed by tandem-mass spectrometry analysis of the eluted peptides and their identification using human proteome databases (7).

What we do know is that there appears to be a poor relationship between mRNA and proteome quantities, a phenomenon that is tissue-specific {Wang, 2019}, and furthermore there is a similarly complex association between proteomic quantity and the detection and quantity of immunopeptides which is also affected by tissue type (9). The non-linear relationship between transcriptome, proteome and immunopeptidome poses challenges for the prediction of immunologically important HLA-I bound cancer-specific peptides.

Some of this discrepancy is potentially due to the effects of protein subcellular compartment distribution, differential protein turnover and proteasomal/immunoproteasomal dynamics.

The HLA-I immunopeptidome is enriched for cytosolic and nuclear proteins, however the ability to bind to HLA-I is the single most significant factor in determining whether an immunopeptide will be detectable. As previously discussed, there are multiple other factors related to antigenic processing which are much more challenging to predict. Immunopeptidomics has, through sheer scale of data generation, greatly improved the prediction algorithms (e.g. NetMHC) that can be used to determine whether peptides will bind HLA (10).However, it cannot accurately determine whether any individual protein will be processed to generate peptides or which of those peptides which contain the correct HLA-binding motifs will be selected for presentation. Thus, as well as helping to improve the HLA binding algorithm accuracy, the empirical immunopeptides identified using immunopeptidomics in tumour studies is invaluable for providing real-world evidence of epitope presentation.

As might be expected, the vast majority of immunopeptides presented are simply self-protein derived and thus immunologically silent, however this technique is now being harnessed to interrogate the T cell epitopes presented on HLA molecules from tumours with the aim of identifying those that might be enhanced for therapeutic use. This review discusses the recent contribution of immunopeptidomics in the pursuit of the identification of CD8+ T cell epitopes in cancer.

**Main body of the article**

HLA-bound peptides on tumour cells could, at least in theory, be derived either from the canonical translation of coding sequence (CDS) or from other processes arising at the genomic, transcriptomic, epigenomic, translational, proteomic and antigen processing levels which lead to sequences absent in the standard reference proteome and are thus non-canonical.

**Canonical CD8 + T cell targets**

Canonical HLA-I bound peptides would, in general, arise from protein sequences predictable from the standard human reference genome. These tumour epitopes can either be identical to those encoded in the host genome, or alternatively can contain tumour-specific mutations which generate amino acid sequences within certain mutated proteins which are unique to the tumour.

Tumour-associated antigens (TAAs) are derived from canonical proteins which are upregulated incertain tumours. Tumour-specific antigens (TSAs; neoantigens) are canonical proteins containing amino acid substitutions arising from non-synonymous SNVs predictable from genome/exome/transcriptome sequencing. Neoantigens can also arise from other genomic events such as indels, RNA splicing events and gene fusions, and these can be predicted using various bioinformatics approaches (11-13). When an amino acid sequence containing an alteration or substitution is presented on HLA and is capable of eliciting an immunogenic response, this is considered a “neoantigen”.

*CD8+ T cell responses to tumour-associated antigens*

It is recognised that, in the hierarchy of desirable anti-tumour CD8+ T cell epitopes, TAAs are lower ranking, simply because they arise from non-mutated canonical proteins, T cells recognising these antigens may be subject to thymic deletion and thus would be less likely than, for example neoantigens, to result in clinically efficacious antitumour activity. Also, to pose no threat following therapeutic intervention, the TAAs would have to be truly specific to the tumour, but as canonical proteins they are often naturally present in specific body regions such as the gonads e.g. in the case of cancer testis antigens (CTAs), where it is assumed that the immune privileged status of the gonads leads to tolerance of TAAs, which can be immunogenic in the tumour. Nevertheless, the challenges associated with finding immunogenic neoantigens using predictive models, and the paucity of direct mass-spectrometric observation of neoantigens, combined with the prevalence of low mutational burden or low HLA-I expressing tumour types, means that TAAs may often offer the only available target. TAAs can be attractive targets since, despite the wide variation in population-wide HLA types, TAA expression per tumour type can be conserved, and therefore offer more predictable epitope targets than neoantigens, even in commonly mutated driver genes. The potential for warehousing TAA peptide-based immunotherapies has been investigated in chronic lymphocytic leukaemia (CLL) (14), a cancer type with low mutational burden. Here the group identified frequent non-mutated CLL-associated antigens which could be recognised by pre-existing T cells in CLL patients offering the potential for off-the-shelf pre-manufactured peptide vaccines which could be assembled based on the characteristics of the individual malignancy. Despite screening for neoantigens, none could be identified in the CLL samples, however several canonical immunogenic CLL-only peptides observed by immunopeptidomics were found to be immunogenic.

An additional class of canonical TAAs could be derived from endogenous retroviruses, an attractive target for immunotherapy since their expression in tumours is positively associated with immune therapy response (15). In a recent study of TAAs in a mouse pre-clinical model of triple negative breast cancer (TNBC), researchers identified, amongst many canonical TAAs, an HLA-I presented endogenous retrovirus derived immunopeptide, within a theoretically non-coding region (16). Although this peptide was weakly immunogenic it provides proof of principle for such elements as in-vivo tumour epitope targets.

*CD8+ T cell responses to tumour-specific antigens*

Recent observations that checkpoint inhibitor response rates are associated with tumour mutational burden are suggestive that CD8+ T cells capable of recognising HLA-I presented peptides containing tumour-specific mutations (so-called neoantigens) and are an important source of CD8+ T cell target in tumours.

Much interest has been focussed on predicting tumour neoantigens using in-silico approaches based on identification of tumour-specific non-synonymous genomic mutations, and using machine learning algorithms to predict which of these might produce translational products capable of binding to HLA-I. Despite the generally accepted accuracy of in-silico HLA affinity predictions, only ~ 6% of purely in-silico predicted neoantigens are immunogenic in functional T cell assays such as IFN-g Elispot (17).

A typical immunopeptidomic neoantigen discovery pipeline involves a survey of cancer-specific mutations in the genome/exome of cells or tissues in order to construct a searchable personalised custom reference database with appended SNV calls, isolation of immunopeptides, mass spectrometric analysis of HLA-I bound peptides using data-dependent acquisition, and searching of the resulting tandem MS spectra against the custom reference databases (Figure 1).

Graphical user interface

Description automatically generated

**Figure 1** **– A typical immunopeptidomics-based neoantigen discovery workflow.** Tumour-specific mis-sense variants are identified by comparative whole exome sequencing of tumour tissue samples. Variant gene transcripts may be confirmed by RNAseq, then neoantigens are predicted using HLA typing data and machine-learning prediction algorithms, and mass-spectrometry proteomics is employed to directly identify HLA-I and -II bound neoantigens in tumour samples. Figure created with BioRender.com.

Very few studies have directly identified neoantigens using this approach, and even fewer have achieved measurements of T cell activation with the discovered neoantigens although the approach has been proven in melanoma (18). Where directly observed neoantigens have been tested, the absence of an immunogenic response can seem surprising, but could reflect either mis-identification or a lack of biochemical/physical difference from the wild type peptide. Various improvements, such as the use of data-independent MS acquisition (19), isobaric peptide tandem mass tag labelling to improve peptide quantitation (20) or ion mobility measurements (21) have shown promise in increasing the sensitivity of immunopeptidomics (measured by the number of observed immunopeptides), but have not yet reached a standardised approach and have not dramatically improved the numbers of validated neoantigens identified to date.

Recent results using immunopeptidomics have demonstrated the paucity of somatic mutation encoded neoantigens in hepatocellular carcinoma (22). As the authors described, the lack of identified neoantigens measured by immunopeptidomics is not evidence of their absence in the HLA ligandome. However, the scarcity of measurable immunogenic neoantigens using the in-silico pipelines is suggestive that, either the majority of neoantigens are not presented, or that the in-silico methods are not sufficiently accurate to use as a reliable guide to the usable list of neoantigens. This lack of direct observation of predicted neoantigens that are immunogenic is evidence of the limited sensitivity of immunopeptidomics for neoantigen discovery approaches.

Examination of the features of the HLA-I peptidome has revealed a trade-off between HLA-I specificity and neoantigen repertoire, indicating the concept that a broader repertoire could provide wider protection from, for example, viral diseases, but peptide repertoire promiscuity of certain HLA-I alleles is associated with worse prognosis after immune checkpoint inhibition (23). The authors postulated that this might be explained by a reduced ability to discriminate neoantigens from self-peptides when patients are carriers of highly promiscuous HLA-I haplotypes, the result of which would be to generate a tolerogenic T cell infiltrating population. Thus, poor anti-viral protection may be a negative trade-off for better antitumour immunity.

**Non-canonical CD8+ T cell epitopes can be TAAs or TSAs**

Various factors contribute to bias in immunopeptidomics studies, including technical issues around the mass spectrometric detection of peptides with extreme biochemical properties. However, it has long been recognised that searching only the canonical proteome for immunopeptides may also miss a number of potential HLA ligands derived from non-canonical sources, the so-called “dark immunopeptidome”. Recent analyses of non-canonical immunopeptides using a monocytic leukaemia cell line demonstrated that mining RNA-Seq data can reveal unconventional peptides apparently derived from noncoding RNA, noncanonical reading frames or untranslated regions (24).

Riboseq is a useful adjunct to RNAseq data in that it provides information on which ORFs are actually translated, which can be used to improve the searchable protein sequence database without over-expansion of the search space. In a recent study, non-canonical HLA-I immunopeptides were assessed in lung cancer using “footprint” evidence from RNAseq and Riboseq data to filter candidates likely to be expressed and presented (25). Over 500 non-canonical (i.e., arising from long non-coding RNAs, UTRs, pseudogenes and transposable elements) immunopeptides were identified in this study of melanoma cell lines and human lung tumour/healthy control tissues. Furthermore, conservation of such antigens between tumours is higher than for neoantigens. However of these, only one immunogenic non-canonical HLA-I immunopeptide (ncHLA-Ip) was identified. This low immunogenicity may arise due to similar issues as for TAAs, namely thymic deletion of the cognate TCR-bearing T cells. Additionally, the authors speculated that the generally low expression of the ncHLA-Ip might reduce the ability of APCs to prime naïve T cells through cross-presentation, and furthermore, this may also limit the engagement of CD4+ T helper cells, reducing CKD8+ T cell help.

The riboseq approach has been used previously to identify translated unannotated open reading frame immunopeptides in the HLA-I immunopeptidome (26). More recently, it has been used to detect multiple thousands of tumour-specific translated novel or unannotated open-reading frames (nuORFs) in melanoma, chronic lymphocytic leukaemia and glioblastoma (27). nuORFs were over-represented in the HLA-I immunopeptidome, consistent with the notion that non-canonical translated products should in the main be unstable and thus usually non-detectable in shotgun proteomic analyses (28), which may pre-dispose them to being processed and presented.

Non-canonical immunopeptides also appear to be more commonly detected in multiple samples in an HLA allele-specific manner than neoantigens. In the same study, riboseq also provided evidential support for the translation of SNVs from whole genome sequencing data, almost a quarter of which were exclusively in nuORFs, and evidence was provided for a nuORF neoantigen able to bind to its predicted allele.

Recent proteogenomic evidence suggests that cryptic proteins arising from non-canonical translational products generate HLA-I peptides more efficiently than canonical proteins and demonstrate a lack of stability and increased disorder that predispose them to entry into the HLA-I pathway (29). The authors suggest that tumourigenesis may create the conditions for increased non-canonical translational events.

Another study, particularly focussing on microsatelite stable and unstable cell lines and biopsy samples, revealed that more than two-thirds of TSAs in colorectal cancer were identified as derived from non-coding regions (30), and were validated using synthetic peptides and in-silico predictions of immunogenicity, but not with T cell assays.

The discovery of so many potential cancer-specific immunopeptides is very exciting and would certainly expand the number of available targets for cancer therapy. The accumulation of immunopeptide databases to catalogue human self immunopeptides in different tissues (such as the HLA ligand atlas project (31) should assist in the identification of TSAs and consequently help to improve cancer immunotherapy.

Challenges in dealing with false discovery rates when using large search spaces in neoantigen discovery studies are gradually being overcome using a combination of deep learning approaches (32, 33) and advancements in de-novo sequencing (34, 35). In the majority of studies to date, synthetic peptide MS spectral alignments are being used as evidence of the validity of the discovered non-canonical immunopeptides, however MS/MS spectral alignment is often not sufficient to prove the identification of non-canonical HLA peptides, and further evidence such as spiking in of isotopically labelled peptides is considered the gold standard in such cases. The clinical significance of these non-canonical epitopes is relatively untested, as immunogenicity was not tested on a large scale in any of these studies. Indeed, immunogenicity analyses are challenging to control for in such circumstances, since the generation of novel peptide sequences with HLA-I binding capability through, for example, peptide splicing events, could generate immunogenic HLA-Ip complexes regardless of whether they are ever naturally presented. There is some discussion over whether HLA-bound peptides identified from immunopeptidomic studies as proteasomal splicing products are in fact derived from other non-canonical translational products (32, 35-37). It is clear that whatever the true origin of non-canonically derived immunopeptides may be, there is a requirement for further analysis of the clinical significance of such entities in cancer therapy.

**Treatments to improve immunopeptidomic analyses of tumours**

Currently, despite recent improvements as discussed above, it is generally accepted that immunopeptidomic analyses lack sensitivity, and that this may partially explain the challenges associated with finding commonly presented peptides in tumours, especially when considering the canonical TAAs with measurable overexpression in multiple patients. Approaches to improve the depth and breadth of the immunopeptidome include treatment with IFN-g, which is known to influence both HLA-I but especially HLA-II expression, particularly in non-APCs. IFN-g treatment has been shown to enhance and alter the triple-negative breast cancer immunopeptidome (38).

There is similar evidence from studies in colorectal cancer organoids where, despite in-silico predictions that 32% of non-synonymous mutations in the coding regions of the genome would result in neoantigens, only 0.5% result in neoantigens detectable by immunopeptidomics (39). The authors instead demonstrated that neoantigens arising from somatic mutations were outnumbered by those arising from noncoding regions and aberrant transcript expression. IFN-g treatment affected both HLA-I and -II immunopeptidomes, notably increasing the HLA-II immunopeptidome size, but did not reveal new neoantigens in either class.

Induction of senescence by treatment with low-dose doxorubicin (below that used to induce immunogenic cell death), enhanced HLA-I processing and presentation, dependent on paracrine self-sustained IFN-I signalling (40). Exposure to a senescent primary human cancer cell line promoted hyperstimulation of autologous tumour infiltrating lymphocytes (TILs) in a neoantigen-specific manner, however further work would be required to examine the broader effect on the TIL repertoire.

Treatment of diffuse large B-cell lymphomas, which often have significantly downregulated HLA-I expression, with chemotherapeutic agents alone or in combination with IFN-g restored HLA-I expression and enhanced HLA-II expression, enabling the identification of novel lymphoma-specific immunopeptides unmasked by the chemotherapy treatment (41). Whilst these novel epitopes were not validated, such an approach is consistent with the notion that chemotherapy enhances anti-tumour immunopeptide presentation, a key part of the principle of ICI therapy combined with neoadjuvant chemotherapy. Whilst no neoantigens were uncovered following chemotherapeutic or IFN-g treatment, the potential remains for such therapeutics to enhance their detection in combination with improved MS sensitivity in the future.

**PTMs as tumour-specific targets in immunopeptidomics**

As the current predictive approaches for detection of both canonical and non-canonical neoantigens rely principally on pre-translational genomic or transcriptomic data, they do not gather information on post-translational modifications. Immunopeptidomic analyses have the capability to examine PTMs, however searches for each additional modification hugely increases the search space, resulting in prohibitively long search times, meaning this area is relatively unexplored. To overcome these challenges, one can either selectively enrich for each modification of interest, or alternatively, improve the database searches of non-enriched immunopeptidomic datasets. One study of note examined PTM-driven motifs in MHC-eluted peptides of selected haplotypes in mouse colorectal cancer (42). The presence of PTMs altered the anchoring positions or inter-anchor regions of the immunopeptides. Extension to a clinical proteomic tumour dataset from a breast cancer cohort revealed cancer specific modifications identical to those found in the mouse model. Perhaps unsurprisingly given the biochemical specificity of HLA haplotypes for determination of peptide motif binding, PTMs were found to alter HLA-I binding preference and TCR recognition. The tumourigenic process may uncover many tumour-associated PTMs, particularly in TAAs, however the mutations giving rise to neoantigens may also give rise to tumour-specific PTMs either directly or through downstream pathway alterations. Thousands of modified peptides were identified in the triple negative breast cancer samples, and several candidate peptides were selectively phosphorylated in the tumours. However, analyses of the phospho-proteome suggested that immunopeptidome phosphorylation were poorly predicted by the proteome data so there remains a reliance on empirical immunopeptidomics data for such studies, and methods to validate the findings require future development.

**Microbial-derived tumour-specific targets in immunopeptidomic studies**

Endogenous retroviruses are DNA sequences in the genome that have been acquired during modern human evolution and have become stable elements of the human genome. They have recently been proposed as potential immunotherapeutic cancer targets as they can generate cancer-specific immunopeptides (43). ERVs provide an abundance of canonical and non-canonical transcription start sites for developmentally regulated genes, many acting as promoters or enhancers, and occupy a space at the interface between self and non-self for both innate and adaptive immunity. In tumours, ERVs can act as alternative promoters in malignant cells, many of which will be interferon-responsive and have immunoregulatory functions. ERVs encoding a HERV-E envelope gene selectively expressed in renal cell carcinomas, identified by transcriptomic analyses, have been demonstrated to produce epitopes which can be presented as HLA-I bound peptides on the surface of tumour cells (44). More recently, a novel mouse ERV antigen was found by differential TAA immunopeptidomics screens in mice (16) and was confirmed as an HLA-I ligand and to be immunogenic, demonstrating anti-tumour immunogenicity during therapeutic vaccination.

Immunopeptidomics also shows promise in elucidating the CD8+ T cell targets during oncolytic tumour therapy. For example, measles virus treatment in experimental glioma treatment induced a type 1 interferon response and a downstream apoptotic cascade accompanied by treatment-induced viral and tumour-associated peptide presentation, features which could be adopted for tailored treatment strategies to improve virotherapy (45). Such approaches have been harnessed to address issues of aggressive relapse following oncolytic vesicular stomatitis virus exposure to generate neoantigens recognising escape phenotype epitopes to specifically target treatment resistance (46).

**Summary**

Neoantigens represent the most desirable CD8+ T cell targets, and a number of clinical trials are underway to assess their utility in different cancer types. Once found, neoantigens are known to have the potential for clinical anti-cancer benefit (47). Surprisingly, in the majority of clinical neoantigen trials to date, vaccines have induced predominantly neoantigen-specific CD4+ T cells and not CTLs, even where HLA-I restricted epitopes have been specifically targeted (47). Since the TMB/ICI relationship appears to hold out, this is suggestive that the neoantigen-specific CD4+ T cell responses are central to the CD8+ T cell antitumour response. Studies in mouse models suggest that immunisation with vaccines targeting a specific neoantigen induce CD4+ T cell responses that potentiate CD8+ T cells targeting other TAAs (or possibly TSAs) (8). Thus, CD8+ T cell cancer epitopes should not necessarily be considered in isolation.

A significant factor in truly understanding the immunogenicity of neoantigens lies in the disadvantages of the current methods of validation. IFN-g Elispot is a useful measure of the immunogenicity of individual peptides when compared to wild type control peptides, however the majority of predictive neoantigen studies use long peptides containing the predicted mutations and do not assess the HLA-restricted peptides, thus leaving the possibility that the true neoantigen target is not properly identified. Furthermore, often the mutations occur in anchor positions of the neoantigens, rendering the wild type peptides redundant as they would not naturally bind and form the HLA-Ip complex. Similarly, non-canonical epitopes may not have properly controlled wild type equivalents that can be checked by Elispot. Results from clinical trials suggest that neoantigen cancer vaccines often engage the naïve T cell repertoire (48). There are unanswered questions relating to the relevance of testing memory T cell responses compared to naïve T cell responses, especially where non-canonical epitopes are concerned.

Current approaches to solve these issues include the examination of clonal expansion of TILs or PBMC-derived CD8+ T cells post-neoantigen stimulation (a measure of memory T cell response) and determination of their cytotoxic activity in killing assays. Whilst this is theoretically ideal, such technology is currently not widely available.

In this review we have examined the role of immunopeptidomics in revealing the potential immunogenic peptides that can be recognised by CD8+ T cells, with the aim of enhancing the activity of these cells to eradicate tumours. It is clear that, whilst immunopeptidomics has revealed much about the various immunopeptides, both canonical and non-canonical, PTMs, pathogen-derived etc, it is currently unclear which, if any, of these potential epitope targets is the most clinically significant.

**Summary points**

* Clinical efficacy of ICI therapy suggests neoantigens are optimum candidates for CD8+ T cell targeted cancer immunotherapy
* Direct immunopeptidomics identification of neoantigens is rare due to current lack of sensitivity of the method
* Tumour-associated antigens can be found conserved in multiple tumour types, but may be weakly immunogenic
* Bioinformatics-based discovery methods looking at non-canonical sources of CD8+ T cell targets are being investigated – limited evidence of robust immunogenicity to date
* The relative contribution of canonical and non-canonical derived epitopes is currently unclear

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