Trends in Immunology

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Review

Molecular mimicry as a driver of T cell-mediated tumour immunity

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Recently, a large pool of antigens derived from viral and bacterial microorganisms showing molecular mimicry with tumour-cell-expressed antigens was identified. These antigens can be presented by MHC molecules and elicit T cells that are crossreactive with microbial antigens and tumour-cell-associated antigens. In the setting of metastatic melanoma, such T cells can contribute to the response induced by immune checkpoint blockade therapy. Here, the current understanding of molecular mimicry in T cell-mediated tumour immunity and how this might be exploited for developing new preventive and therapeutic approaches for cancer is described. In particular, the literature on the concept and evidence of molecular mimicry in cancer is reviewed, covering the whole translational spectrum, from the antigen discovery strategy to the clinical evaluation.

Highlights

Several peptide sequences derived from pathogenic and non-pathogenic microbes, so called microbial-associated antigens (MAAs), show high sequence homology with canonical and noncanonical tumour-associated antigens (TAAs).

T cells crossreactive with MAAs and TAAs showing homology (MAA/TAA pairs) are identified in both healthy individuals and cancer patients confirming that exposure to microbes may induce a preventive anticancer T-cell repertoire.

Structures of T cell receptors (TCRs) crossreactive with epitopes derived from microbes and cellular self-antigens have been resolved, proving that the same TCR elicited by a MAA binds the homologous TAA.

Epitopes from intratumoral bacteria can be presented by MHC class I and II molecules and elicit tumour-infiltrating lymphocytes targeting TAAs.

Phase 1 clinical trials are currently evaluating the safety and immunogenicity of cancer vaccines based on TAA-homologous MAAs.

Significance

The molecular mimicry has a twofold relevance. First, at an individual level, exposure to a large set of microbial-associated antigens MAAs during the course of a lifetime will increase the chance of establishing a broad preventive repertoire of memory $CD8^+$ T cells able to promptly crossreact with mimicking TAAs (natural anticancer vaccination). Secondly, at a general level, non-self MAAs with high homology to shared self TAAs highly expressed across several cancer types may be employed in multitargeting preventive as well as in therapeutic cancer vaccines.

Self versus non-self-antigens and immune crossreactivity

Immunological tolerance prevents potentially harmful immune responses against our own (self) antigens and also against beneficial or harmless non-self-antigens, such as those derived from microbiota, food, and the environment [1]. However, B cell or T cell immune responses can develop and inadvertently attack self-antigens [2]. There are several reports of B cells that have been originally elicited by microorganism-associated antigens, crossreacting with self-antigens and causing autoimmune disease [3–5]. Nowadays, **molecular mimicry** (see Glossary) is defined as a similarity in structure of full-length or short stretches of proteins with or without sequence homology [6,7].

Only sporadic evidence has been reported of microbe-derived antigens showing molecular mimicry of tumour antigens [8]. In recent years, the field has been significantly boosted by a number of novel findings and reports proposing extensive conformational and immunological evidence for a large pool of **microbial-associated antigens (MAAs)** mimicking **tumour-associated antigens (TAAs)**, which may shape antitumour immune responses and impact cancer therapy [9].

In this review, the bioinformatics, structural, and immunological experimental evidence for molecular mimicry between MAAs and TAAs is comprehensively reported on. This includes recent clinical evidence, which has provided substantial support to the biological relevance of molecular mimicry in cancer immunology [10].

Identifying molecular mimicry

The quest for sequences with potential molecular mimicry and antigenic relevance ought to be restricted to small peptides recognized by the immune system [11]. Moreover, identical short peptides (<6 amino acids) from paired proteins should be analysed in the context of the full sequence (8–15 amino acids) covering the entire sequence binding the MHC molecule. Molecular mimicry may occur only if the similar/identical sequences are perfectly aligned along the sequences of two epitopes presented by the same HLA allele. A single amino acid shift, or a single

amino acid insertion/deletion or a predicted affinity to different HLA alleles will eliminate the mimicking effect (Box 1). This is the only strategy that ensures an accurate prediction of binding to the same HLA haplotype of the peptides derived from humans and microorganisms and resulting in a molecular mimicry. Indeed, according to the position of the changed residues along the peptide sequence, the binding affinity of the epitopes to the same HLA can be affected at a different scale. As general rule, if the sequence difference will occur at the residues involved in the binding to the HLA, very likely the two epitopes will not be presented by the same allele and there will be no molecular mimicry effect (Figure 1).

Promiscuous antigen recognition by lymphocytes

Unlike the B cell receptor (BCR) and circulating antibodies, which recognize their target epitopes in the context of the entire protein, T cells can recognize their cognate proteinaceous epitopes via the T cell receptor (TCR) when the appropriate fragmented peptide is presented by the cell surface MHC class I and II molecules [12].

It has been estimated that $<10^8$ naive T cells are circulating in an individual; a number largely insufficient to recognize the theoretical $>10^{15}$ peptides presented by the MHC molecules (peptide-MHC, pMHC) [13]. Since TCRs, unlike BCRs, do not undergo post-translational affinity maturation by somatic hypermutation. only a high level of degeneracy in the TCR–pMHC interaction may overcome such a limitation. It has been proposed that each TCR may recognize multiple different MHC-bound peptides (up to 10^6) (**TCR–pMHC binding promiscuity**) [14], protecting the organism from virtually all pathogens and cancer cells (Figure 2A–F).

Molecular interactions between TCR and peptides

Several studies have focused on understanding the structural basis of TCR recognition of peptide–MHC complexes [11,15] (Box 1). As the contact zone between the TCR and peptide is focused on the core **TCR-facing residues** (four or five amino acids), there is the increased

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Box 1. Technical details about the strategy for searching homology between MAAs and TAAs and mechanisms of interaction between TCRs and pMHCs

Parameters for MAA/TAA homology search

The number of proteins with similarity between humans and microorganisms appears to be strictly dependent on the stringency parameters (% of sequence coverage and identity). The higher the stringency parameters (sequence coverage >70% and identity >70%), the lower the number of similar proteins between humans and other organisms [57]. Similarity in protein sequences may reflect an evolutionary relationship, which is expressed by the expect value (E value), the number of times a sequence alignment with a certain score or better is expected to occur by chance in a database search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). In particular, high E values ($>10^{-10}$) are indicative of a random homology, while low E values ($<10^{-80}$) are indicative of highly possible evolutionary relationships.

Mechanisms of TCR–pMHC interactions

In order to have an immunological relevance, the homologous MAAs and TAAs identified according to the parameters described above should be recognized by the same TCR. TCR binds in an end-to-end manner, sitting atop the peptide–MHC platform. Here, typically, the TCR α-chain and TCR β-chain is positioned over the α2-helix and α1-helix of MHC-I (β-chain and α-chain of MHC-II), simultaneously co-contacting the composite surface of the MHC and peptide. This consensus docking topology is observed for most solved TCR–pMHC complexes, leading to the concept that TCRs may have an inherent germline bias for MHC molecules [58]. However, reversed TCR–pMHC complexes have been described for both MHC-I and MHC-II molecules [59,60], indicating that co-receptor/LCK signalling constraints are the principal determinant underpinning a consensus docking topology [61]. While the TCR–pMHC interface is large, the affinity of the interaction is typically weak (in the μM range), in stark contrast to antibody–antigen interactions [15]. The TCR generally makes most contacts with the MHC surface – often, but certainly not exclusively, being mediated by the CDR1 and CDR2 loops of the TCR. In contrast, typically 1–3 amino acids from the peptide that protrude out of the MHC cleft interact with the TCR, and these interactions are frequently, but not always mediated by the hypervariable CDR3 loops of the TCR [15].

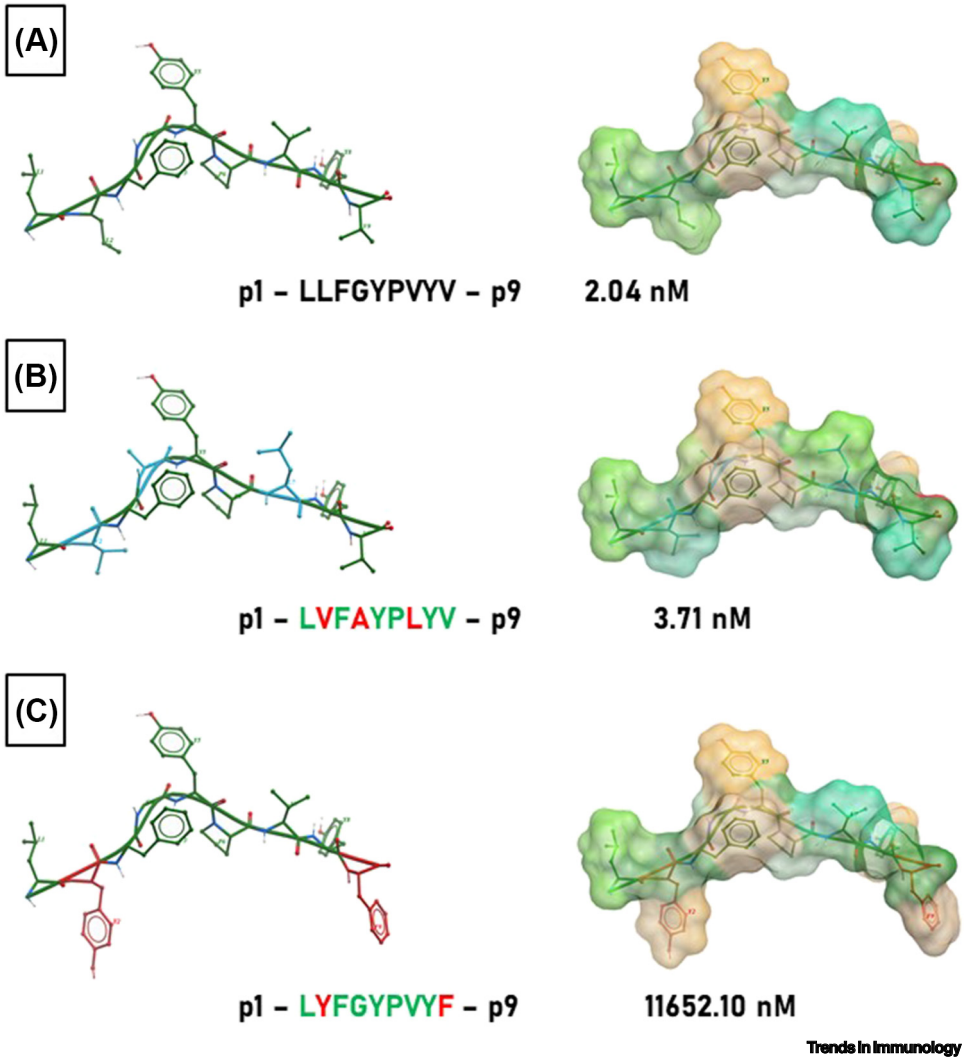

Figure 1. Impact of residue differences on the affinity to HLA alleles. Examples of different changes in the epitope sequences and their impact on the affinity to HLA-A*02:01 allele. (A) Reference epitope sequence with the reported affinity and conformation. (B) Changes in positions 2, 4, and 6 do not alter neither the affinity to the HLA allele nor the conformation of the epitope. (C) Changes in positions 2 and 9 completely abrogate the affinity to the HLA allele, inducing a relevant modification in the binding residues.

Glossary

Microbial-associated antigen (MAA): non-self HLA-restricted antigen derived from pathogenic and non-pathogenic microorganisms.
Molecular mimicry: similarity between non-self antigens expressed by infectious microorganisms and self-antigens on human normal or cancer cells, able to elicit crossreacting immune response.
TCR-facing residues: amino acid residues at the core of the antigen reacting with the TCR αβ chains when presented by the pMHC complex.
TCR–pMHC binding promiscuity: the ability of the same TCR to recognize a broad spectrum of epitopes with a limited sequence overlap when presented by the HLA.
Tumour-associated antigen (TAA): self-HLA-restricted shared antigen overexpressed by cells of a specific cancer type across patients.
Tumour-infiltrating lymphocytes (TILs): T lymphocytes specific for tumour antigens infiltrating the tumour microenvironment.

ability of any given TCR to crossreact towards any epitope that displays sequence homology to each other, including cancer antigens [16,17] (Figure 3).

Recently, an MHC-independent binding of epitopes within intact proteins by αβTCRs has been described, broadening the modalities of recognition and interaction between T cells and proteins [18,19]. Such antibody-like recognition expands the current dogma of the T cell-mediated interaction with epitopes only when complexed with MHC. This would suggest a broader spectrum of antigenic molecular mimicry occurring also without the HLA restriction, with possible crossallelic implications. Moreover, it would increase the anticancer therapeutic efficacy, overcoming the tumour immune evasion associated with the loss of MHC class I antigen presentation [20].

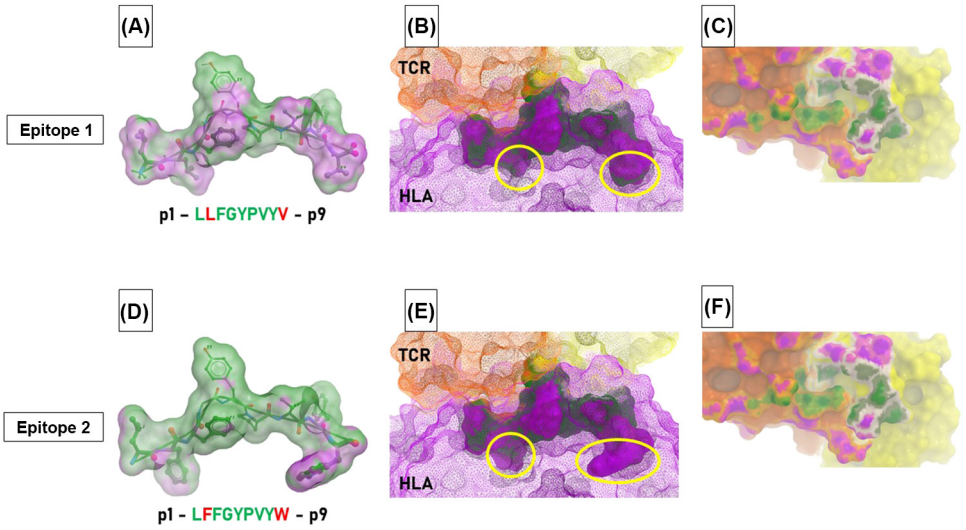

Figure 2. T cell epitopes showing identical contacts with T cell receptor. Example of two T cell epitopes sharing the identical core p3–p8 residues in green (A and D). The different amino acids at the p2 (L vs F) and p9 (V vs W) positions do not significantly affect the interaction with the HLA-A*02:01 molecule (B and E). Moreover, the contact areas of both epitopes with TCR α and β chains are identical (green areas) (C and F).

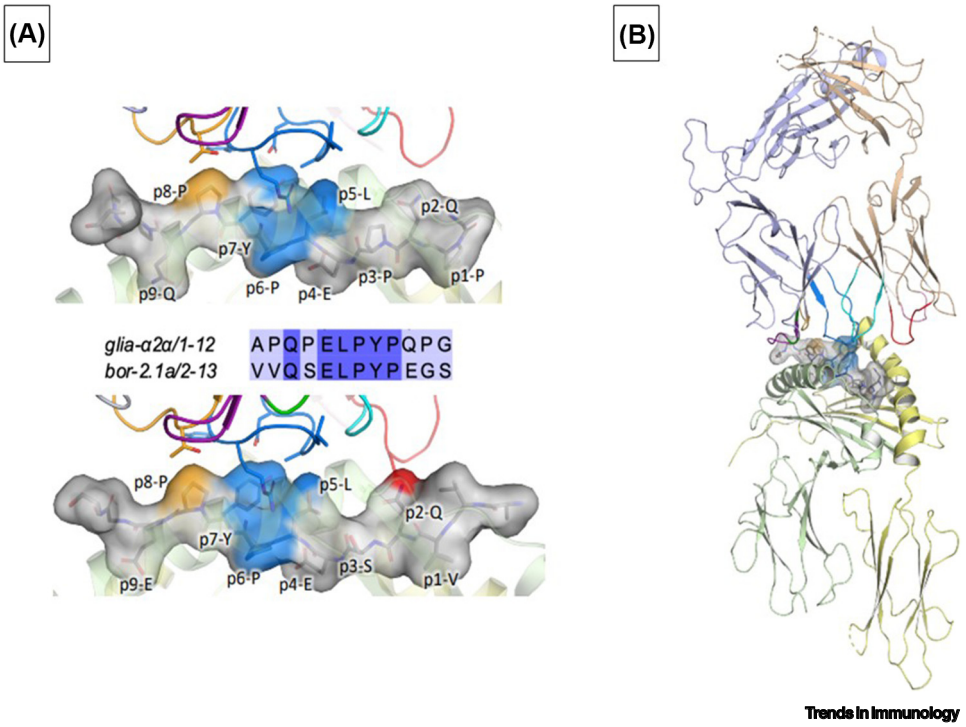

Figure 3. T cell receptor (TCR) crossreacting with antigens showing molecular mimicry. T cell epitopes from gliadin and a microbe sharing the identical core p3–p7 ELPYP residues. (A) The different flanking residues do not interfere with the crossreactivity of the same TCR. (B) Structural analysis confirms the binding of the two epitopes by the same TCR.

Molecular mimicry and T cell crossreactivity with cancer antigens

Consequent to the described mode of interactions between epitopes and TCRs, T cells may crossreact with several self and non-self pMHC complexes, resulting in a broad immunological memory. In such a perspective, individuals could undergo a natural preventive vaccination against cancer every time they are exposed to pathogens as well as in response to exposure to the microbiota [21]. Memory $CD8^+$ T cell clones specific for the MAAs would be promptly recalled by a heterologous highly similar TAA expressed by cancer cells in a nascent tumour lesion. Such non-self MAAs do not suffer from immunological tolerance and would be strategic for anticancer vaccines, overcoming the limited immunogenicity of TAAs [9,21] (Figure 4).

Crossreactivity in melanoma, lung and liver cancers

The melanoma-associated antigen recognized by T cells 1 (MART-1) AAGIGILTV epitope was the first TAA to be reported as mimicking ubiquitous herpes simplex virus (HSV)1- and HSV2-derived epitopes [22]. $CD8^+$ T cells reactive to the Melan-A/MART-$1_{27\text{-}35}$ was identified in both HLA-A*02:01^+ healthy donors and melanoma patients [23]. A molecular mimicry in patients with melanoma has also been described for MAGE-A6 HLA-A2*02:01-restricted peptides and a peptide from the HF-2 permease protein of *Mycoplasma penetrans* [22]. In both HLA-A*02:01^+ healthy donors and melanoma patients, the stimulation induced by the HF-$2_{220\text{-}229}$ was significantly greater compared to the MAGE-$A6_{176\text{-}185}$, confirming the *in vivo* priming by the *M. penetrans* [24].

Tumour-infiltrating lymphocytes (TILs) in human lung cancer have been shown to crossreact with an antigen derived from the TMEM161A protein, which is a TAA overexpressed in non-small cell lung cancer (NSCLC), and an antigen derived from the Epstein–Barr virus (EBV) latent membrane protein 2a (LMP2a) [25]. An HLA-A*02:01-restricted peptide from the tail length tape

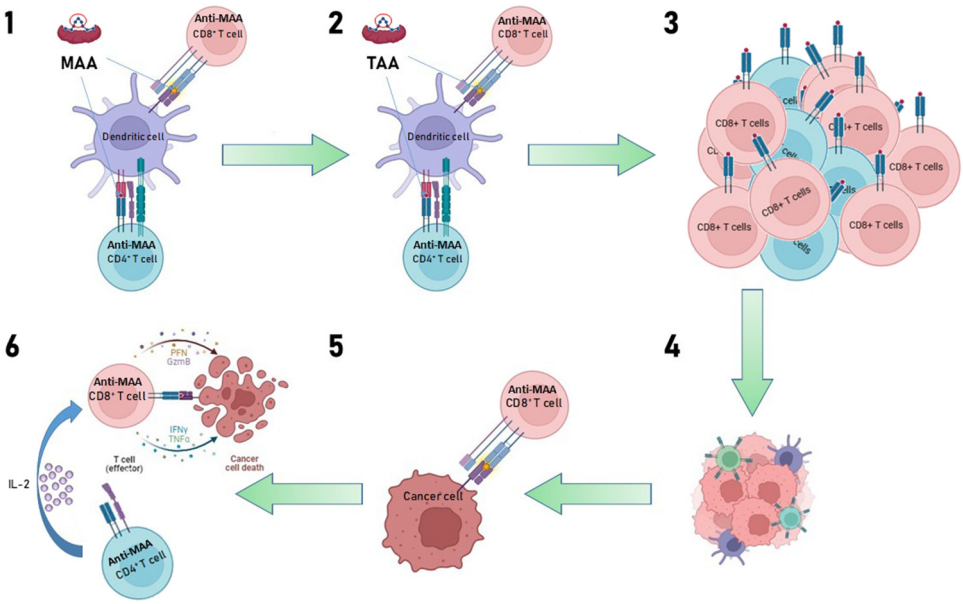

Figure 4. Mechanism of T cells crossreactivity induced by molecular mimicry. (1) Microbial-associated antigens (MAAs) prime $CD4^+$/$CD8^+$ T cell immunity, establishing a memory immunity. (2) A similar TAA (molecular mimicry) is expressed by cancer cells and promptly recognized by the MAA-primed T cells. (3) Crossreacting $CD4^+$/$CD8^+$ T cells are greatly expanded and (4) efficiently infiltrate the tumour at early stage. (5) MAA-specific $CD8^+$ T cells bind TAAs expressed by cancer cells and (6) kill cancer cells in collaboration with the $CD4^+$ T helper cells. Figure created with BioRender.

measure protein (TMP) of a prophage found in the genome of the bacteriophage *Enterococcus hirae* has been described that shares high homology with a peptide from glycerol-3-phosphate dehydrogenase 1-like (GPD1-L), which is another TAA associated with NSCLC. The TMP peptide is able to stimulate $CD8^+$ T cells ex vivo from both healthy individuals and NSCLC patients [26].

Mutated neoantigens in patients with primary hepatocellular carcinoma (HCC) share high sequence homology with MAAs. Molecular mimicry between three mutation-derived neoantigens and MAAs were identified in a single patient with HCC who showed the longest overall survival in the cohort [27]. Subsequently, novel HLA-A*02:01-restricted or HLA-A*24:02-restricted epitopes from proteins specifically overexpressed in HCC have been predicted and validated [28]. A subset of these TAAs show a marked homology to HLA-A*02:01 or HLA-A*24:02-restricted peptides derived from hepatitis C virus, adenovirus, hepatitis B virus, and influenza A virus. The paired epitopes were validated *in vitro* for binding affinity, stability and T cell crossreactivity was confirmed by *ex vivo* experiments [28].

Recent findings in molecular mimicry in cancer

In recent years, the quest for a broader implication of the molecular mimicry in cancer has gained momentum with the description of several MAAs mimicking both characterized TAAs as well as antigens derived from proteins overexpressed in specific cancers. Indeed, in addition to several well characterized known TAAs (https://caped.icp.ucl.ac.be/peptide/list), a larger number of non-canonical TAAs may be predicted to be presented by cancers from overexpressed proteins. Therefore, the search for mimicry has been performed taking into account both types of TAAs.

Viral-derived MAAs and known TAAs

An unbiased BLAST search for molecular mimicry between sequences from human viruses and all TAAs available at cancer peptide database returned a pool of viral epitopes with the appropriate characteristics [29]. Twenty viral sequences homologous to TAAs were predicted to be strong binders (e.g. <100 nM affinity) to the same corresponding MHC class I alleles. Among these, the *MLGTHAMLV* peptide from the membrane protein UL20 of HCMV was found highly homologous to the *MLGTHTMEV* epitope from the melanoma-associated antigen gp100 [30]. In addition, the *KMDEEHPGL* epitope derived from the Pol PB1 protein of influenza B virus is highly homologous to the *KMDAEHPEL* Secernin 1 peptide, a TAA associated with gastric and colorectal cancers [31]. Surprisingly, 45% of the mimicking sequences identified are derived from HIV-1 proteins, with high homology to consistently proven TAAs such as carcinoembryonic antigen (CEA), tyrosinase, gp100, and telomerase. The *IMVGALIGV* epitope derived from the HIV-1 env protein is highly homologous to the *IMIGVLVGV* CEA peptide, which has been previously reported to be recognized by peripheral blood mononuclear cells (PBMCs) from healthy donors [32]. This could explain epidemiological data showing that people living with HIV/AIDS (PLWHA) have a lower incidence of non-AIDS-related tumours [33]. All the reported MAA/TAA sequence homologies have been further supported by similar peptide conformation which directly correlates with the PBMC crossreactivity in *ex vivo* immunological evaluations [29].

Microbiota-derived MAAs and known TAAs

The same unbiased homology search for molecular mimicry has been more recently conducted between the publicly available HLA-A*02:01 and 24:02-restricted TAAs and peptides from microbiota species of the Firmicutes and Bacteriodetes phyla, which together account for >90% of the human gut microbiota [34,35]. Overall, 619 restricted-TAA-like peptides have been identified in both phyla, but only among the HLA-A*02:01-restricted were predicted epitopes at high affinity (<10 nM) (95 from Firmicutes and 55 from Bacteroidetes) and MAGE-A10 is the TAA with the largest number of homologous peptides in both phyla with high affinity (30 in total). The

consensus of the microbiota-derived epitopes confirms that the matched epitope sequences are not specific to a single genus. Strikingly, three microbiota-derived peptides showed a sequence identical to the corresponding TAA.

Paired peptides show the TCR-facing core residues in a spatial conformation which may be significantly similar if not identical, suggestive of a crossrecognition by T cells. An example is reported for MAGE-A3 for which 48 homologous peptides derived from both Bacteroidetes and Firmicutes phyla have been identified and overlapped. A subset of ten MAAs show a highly similar conformation to the MAGE-A3, corresponding to a high conservation of amino acid residues at each position along the peptide sequences [34]. In support of such prediction and bioinformatics data, we have recently shown that $CD8^+$ T cells reactive to MAAs derived from microbiota and their homologous TAAs are identified in both healthy individuals and in patients with cancer [36]. $CD8^+$ T cells from both healthy individuals and cancer patients recognizes microbiota-derived MAAs, confirming that indeed they are naturally presented to the immune system in association with MHC class I molecules. In particular, most of the subjects in the two experimental groups show circulating PBMCs reactive against a peptide derived from *Bacteroidetes bacterium* that is highly homologous to a peptide from MAGE-A3/12, and against a peptide derived from *Eubacterium* sp. that is highly homologous to a peptide from MAGE-C2. Reactivity against TAAs was observed in both groups, and healthy individuals showed reactivity against MAGE-A1 and MAGE-C1 TAAs, suggesting a priming due to a molecular mimicry with microbiota MAAs. The biological relevance of such reactivity was further confirmed by crossreactive CTL activity, strongly suggesting that pre-existing antimicrobial $CD8^+$ T cells may play a role in controlling tumour growth [36,37].

HIV-derived MAAs and noncanonical TAAs

The homology and the molecular mimicry between MAAs and TAAs has been described recently also for noncanonical TAAs [38,39]. The molecular mimicry may play a key role in PLWHAs from non-AIDS-defining cancers, namely breast, prostate, and colon cancers [40]. Predicted HIV-1 epitopes with high affinity to different HLA allomorphs share high homology to peptides restricted to the same alleles and predicted from tumour-specific proteins (CEACAM8, CDH17, GLOD5, PPM1E, TRIM16, and EPCAM for colon cancer; CCR9 for breast cancer; and NDUFS2 for prostate cancer). The strongest evidence for molecular mimicry was observed between the HLA-A*02:01 restricted peptides derived from the Vpu protein and a peptide derived from the EPCAM protein (colon cancer). Similarly, an HLA-A*02:01-restricted peptide and an HLA-A*24:02-restricted peptides derived from the Env protein of HIV show strong evidence for molecular mimicry with the two peptides derived from the CCR9 (which is a TAA that is associated with breast cancer). For both cases of molecular mimicry, spontaneous reactivity against the predicted TAAs was observed only in HIV-positive subjects supporting the notion that T cell priming by HIV epitopes induces a tumour-specific protective immunity [38].

SARS-CoV-2-derived MAAs and noncanonical TAAs

Homology between SARS-CoV-2-derived antigens and publicly available TAAs has been recently reported by our group [39]. HLA-A*02:01 restricted epitopes derived from all the variants of concern were identified along the entire SARS-CoV-2 proteome, within and outside the protein sequences present in the vaccine. Several examples of antigens that share a high degree of sequence and conformational homology with TAAs (particularly melanoma TAAs) have been observed. Two ORF1ab-derived peptides and one nucleocapsid-derived peptide show sequence homology to two different TAAs. Likewise, some of the TAAs show homology with more than one SARS-CoV-2 epitope. For instance, a PRDX5-derived peptide shows homology with 5 SARS-CoV-2-derived peptides and an MDK-derived peptide shows four homologies. The

reactivity to each epitope is highly individual, although a consistent reactivity to all SARS-CoV-2 and TAA epitopes is observed, with an average of 0.81% crossreactive T cells. In particular, the highest reactivity is observed for the ORF1ab-derived VLLSMQGAV/ MDK-derived ALLALTSAV and the MEMBR-derived KLLEEWNLV/ HER-2-derived RLLQETELV epitope pairs, reaching >9% and ~6% of the total circulating T cells, respectively. Therefore, also in the case of SARS-CoV-2 infection, molecular mimicry between viral epitopes and TAAs may possibly elicit crossreactive memory T cells with the potential to contribute to anticancer immunity. Confirmation of such predictable protective effect will be provided by the incidence and progression of specific cancer types in the next years in the general population.

Molecular mimicry and immune checkpoint blockade

The efficacy of cancer patients' treatment with immune checkpoint blockade (ICB) has been shown to be influenced by gut microbiome composition [41,42]. This would suggest that, in a multifactorial context, any pre-existing immunity directed against commensal-derived peptides sharing structural homology with TAAs might contribute to the overall clinical outcome.

Recently, this hypothesis was confirmed in a clinical setting, demonstrating the relevance of specific bacterial peptides derived from flagellin-related genes that mimic the structure of some melanoma TAAs [10]. Importantly, because of their stable presence in the gut of patients with melanoma experiencing a complete response to ICB, this set of TAA-mimicking MAAs [which we named flagellin-related genes of Lachnospiraceae (FLach) peptides] hold exceptional prognostic value and enable efficient stratification of patients undergoing immunotherapy. Reactivity against FLach peptides is preferentially detected in PBMCs (isolated prior to ICB therapy) and in TILs from patients responding to ICB therapy. These FLach peptide-reactive T cells can enhance antitumour immunity, and could be useful as adjuvants for cellular and ICB immunotherapies. The overall value of this model is supported by preliminary evidence emerging from independent clinical and preclinical studies across various tumour types (for example, NSCLC, renal cell carcinoma, and glioblastoma) [43].

Intratumoral MAAs and molecular mimicry

Beyond intestinal and skin microbiota, the role of intratumoral bacteria and fungi in cancer progression and immune surveillance has been extensively studied in recent years [44]. The percentage of tumour samples positive for bacterial DNA ranges from 14% (in melanoma) to 63% (in breast cancer). Bacteroidetes, Firmicutes, and Proteobacteria are the most abundant intratumoral phyla with variable ratios between tumour types. The clinical impact of the intratumoral microbiota greatly varies from fostering the tumorigenesis to influencing therapeutic responses, particularly in chemotherapy and immunotherapy [45,46]. MHC class I- and II-restricted peptides derived from intratumoral bacteria present in melanoma, glioblastoma, and colorectal cancer were recently described and recognized by TILs from the same patients [47].

In addition, within the scope of the present review, intratumoral bacteria may present antigens that show a molecular mimicry with TAAs and induce crossreactive antitumour T cell responses. A recent study identified MHC class II peptides derived from intratumoral bacteria in glioblastoma and showed that TILs and also peripheral T cells specific for bacteria and microbiota-derived peptides crossreact with tumour-specific and tumour-associated glioblastoma antigens in an autologous setting [48]. Analysis of core-binding motifs in this study showed that amino acid preferences at HLA-DR anchor positions were similar between the bacterial and tumour peptides, but different amino acids were present at nonanchor positions [49].

Current evaluation of the molecular mimicry strategy in clinical trials

The bulk of the bioinformatics, immunological, and structural information supporting the MAA/TAA molecular mimicry represents the rationale of using MAAs as a cancer vaccine for eliciting a T cell response crossreacting with tumour cells expressing homologous TAAs (https://www.enterome.com/clinical-trials/). The SIDNEY (EONHL1-20) is an active nonrandomized Phase 1/2 trial of a cancer vaccine as a monotherapy and in combination with lenalidomide and/or rituximab for the treatment of patients with indolent non-Hodgkin lymphoma (iNHL). The vaccine includes 4 HLA-A*02:01-restricted MAAs that show molecular mimicry with the B cell-expressed antigens CD20, CD22, CD37 and CD268 (the BAFF receptor) (Clinical trial information: NCT04669171) [50].

The ROSALIE (EOGBM1-18) is a completed nonrandomised Phase 1/2 cancer vaccine trial in combination with an immune checkpoint inhibitor (nivolumab, Opdivo®) plus or minus bevacizumab for the treatment of patients with first progression/recurrence of glioblastoma. The vaccine includes 3 HLA-A*02:01-restricted MAAs displaying molecular mimicry with glioblastoma-derived TAAs (namely IL-13Rα2, BIRC5, and FOXM1) (clinical trial information: NCT04116658) [51]. The same formulation was evaluated in the SPENCER (EOADR1-19) trial. The latter was a randomised Phase 1/2 study of the vaccine in combination with the immune checkpoint inhibitor nivolumab for the treatment of patients with locally advanced or metastatic adrenocortical carcinoma or malignant pheochromocytoma/paraganglioma [52]. The study was prematurely terminated by the Sponsor as a strategic decision (clinical trial information: NCT04187404). The AUDREY (EOCRC2-22) is an active not-recruiting nonrandomised open-label phase 1/2 cancer vaccine trial in combination with an immune checkpoint inhibitor (nivolumab, Opdivo®) for treatment of patients with unresectable, previously treated, metastatic colorectal cancer. The vaccine includes 5 HLA-A*02:01-restricted MAAs mimicking five TAAs (namely, BIRC5, FOXM1, UBE2C, CDC20, and KIF2C) (clinical trial information: NCT05589597) [53]. All the cancer vaccine formulations in the clinical trial include universal cancer peptide 2 (UCP2), which is a $CD4^+$ T cell recognized peptide derived from the human telomerase reverse transcriptase catalytic subunit (hTERT).

All these trials aim at assessing safety, tolerability, immunogenicity, and preliminary efficacy of the treatments. Results published as meeting abstracts show a promising safety and immunogenicity profile when using molecular mimicry for cancer vaccine [50–53]. In the 322 patients enrolled in the four trials, no related grade ≥3 adverse events were seen. Expansion of $CD8^+$ T cell populations showing reactivity against MAAs and mimicking TAAs (crossreactivity) was detected in all responding patients. Moreover, promising initial signs of clinical efficacy have been observed in all four trials. In particular, the SIDNEY study has suggested clinical activity in 4/9 patients as vaccine monotherapy (one partial response, one size reduction 15%, one 20% reduced tracer uptake, and one 5/6 target lesions reduced metabolic activity) compared to an overall objective response in 6/9 patients and a complete response in 5/9 patients, when the cancer vaccine is combined with lenalidomide and/or rituximab [50].

Concluding remarks

The molecular mimicry is relevant in shaping the antitumour T cell repertoire (Table 1). The repertoire of memory $CD8^+$ T cells elicited during life by MAAs mimicking TAAs may be promptly recalled by cancer cells and inhibit or delay tumour growth in its early stage of development (natural preventive cancer vaccine).

All the reported examples of molecular mimicry between MAAs and TAAs represent an unprecedented opportunity to develop next-generation off-the-shelf therapeutic vaccines for cancer.

Outstanding questions

Can we identify a memory T cell repertoire naturally elicited by microbes and crossreacting with TAAs which plays a real role in controlling tumour growth or progression?

Can we identify a T cell signature predicting the individual susceptibility to cancer?

Can we select TAA-homologous non-self MAAs for developing safe and immunogenic preventive and/or therapeutic cancer vaccines?

Can these cancer vaccines be applied to multiple cancer types expressing the same TAA?

Is it feasible to develop oral probiotics including specific phyla for eliciting MAA-specific T cells crossreacting with TAAs?

Table 1. Examples of homology between TAAs and MAAs

TAAs			MAAs		Refs
TAA peptide	TAA amino acid sequence	Relevant cancer	Microbial source of MAA	MAA amino acid sequence	
MART-1_{27-35}	AAGIGILTV	Melanoma	HSV-1	GIGIGVLAA	[62]
			Escherichia coli	QAGIGILLA	
MART-1_{26-35}	EAAGIGILTV	Melanoma	Pseudorabies virus	VIAGIGILAI	[63]
MAGE-$A6_{172-187}$	HVYIFATCL	Melanoma	*Mycoplasma penetrans*	YIYIFAACL	[25]
$TMEM161A_{437-445}$	ALGGLLTPL	NSCLC	EBV	CLGGLLTMV	[26]
GPD1-L_{30-38}	KLQKFASTV	NSCLC	*Enterococcus hirae*	KLAKFASVV	[26]
MDK_{7-15}	LLLTLLALL	multiple	Adenovirus	LLLTLLLLL	[29]
$Gp100_{178-187}$	MLGTHTMEV	melanoma	HCMV	MLGTHAMLV	[29]
MAGE-$A1_{278-286}$	KVLEYVIKV	multiple	*Firmicutes bacterium*	KVLEYVIRV	[34]
MAGE-$A12_{271-279}$	FLWGPRALV	multiple	*Bacteroidetes bacterium*	FLWGSIALV	[34]
MAGE-$C1_{1083-1091}$	KVVEFLAML	multiple	*Clostridium bacterium*	KILEFLAML	[34]
$EPCAM_{280-287}$	VVAGIVVLV	Colon ca	HIV-1	VVAGIIALV	[38]
$GLYPICAN3_{144-152}$	FVGEFFTDV	Liver ca	SARS-CoV-2	FVNEFYAYL	[39]

Identical residues between TAAs and MAAs are underlined.

In particular, MAAs with high homology to shared TAAs highly expressed across several cancer types may be used in multitargeting preventive as well as in therapeutic cancer vaccines. The inclusion of preventive strategies based on MAAs in paediatric/adolescent vaccine programmes could establish crossreactive memory T cell populations that can be recalled at early stages of tumour growth, thereby providing an increased chance of immune control. In the immediate future, MAA-based vaccines could be implemented as a preventive strategy in populations at high-risk for cancer development (for example, patients with cirrhotic liver or with cervical intra-epithelial neoplasia).

Besides the limitations associated with all immunotherapeutic strategies (vaccines and T cell therapy), specific drawbacks should be carefully taken into consideration regarding this proposed approach. One is the possible immunological tolerance established against such MAAs, which could represent an escape mechanism for cancers expressing the homologous TAAs. Indeed, viral acute infections induce highly effective antiviral T cells. On the contrary, viral persistence is associated with the development of functionally exhausted T cells that are unable to elaborate typical antiviral effector functions [54]. Similarly, microbiota may elicit an appropriate or an inappropriate immune education, according to the moment in life of colonization, greatly impacting on the susceptibility to diseases in later life [55]. In particular, the disease risk may begin at the earliest days of life, including the antenatal period. It has been shown that the maternal microbiota are able to ready neonatal innate immunity starting from pregnancy. This will improve the shielding capacity of the intestinal wall against microbial penetration after birth [56]. In both cases, MAA-specific T cell clones may be functionally impaired, with a consequent inability of responding to the homologous TAA. Therefore, the positive or detrimental effect of T cells potentially crossreactive with the homologous MAAs and TAAs needs to be experimentally proven. The currently ongoing Phase 1/2 clinical trials of cancer vaccines based on MAA and TAA crossreactivity will provide a critical ground of knowledge for building and expanding upon this strategy (Clinical trial information: NCT04669171; NCT05589597).

In addition, considering that tumour antigens targeted with the proposed approach are self-antigens, caution about the potential induction of autoimmunity will be needed. Vaccination with highly antigenic and immunogenic MAAs could elicit an efficient T cell response potentially crossreacting with similar self-antigens, other than TAAs, and result in a pathogenic autoimmunity. However, considering that the MAAs are derived from microorganisms naturally present in the ecosystem surrounding human beings, the risk of inducing autoimmunity should be minimal or no greater than what experienced in real life (see Outstanding questions).

Finally, upon confirmation of the T cell crossreactivity for each MAA/TAA pair, this strategy could represent a turning point in the preventive and therapeutic cancer vaccine field, allowing the development of safe and convenient off-the-shelf vaccines for the treatment of a vast number of patients affected by the same or different cancers.

Author contributions

All authors contributed to drafting and revising the manuscript. LB coordinated the entire project.

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Declaration of interests

The authors declare no potential conflicts of interest.

Data availability

Data and material are publicly available at https://doi.org/10.5281/zenodo.16417662.

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