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Original research

Combination strategies to durably suppress HIV-1: Soluble T cell receptors

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A B S T R A C T

Immunotherapeutic interventions to enhance natural HIV-specific CD8^{+} T cell responses, such as vaccination or adoptive T cell transfer, have been a major focus of HIV cure efforts. However, these approaches have not been effective in overcoming viral immune evasion mechanisms. Soluble T cell receptor (TCR) bispecifics are a new class of 'off-the-shelf' therapeutic designed to address these limitations. These biologics are built on the Immune mobilising monoclonal TCRs against X disease (ImmTAX) platform, which was pioneered in oncology and recently validated by the FDA's approval of tebentafusp for treatment of metastatic uveal melanoma. ImmTAV® are an application of this technology undergoing clinical development for the elimination of chronic viral infections. ImmTAV molecules comprise an affinity-enhanced virus-specific TCR fused to an anti-CD3 effector domain. Engineering of the TCR confers extraordinary specificity and affinity for cognate viral antigen and the anti-CD3 enables retargeting of non-exhausted cytolytic T cells, irrespective of their specificity. These features enable ImmTAV molecules to detect and kill infected cells, even when expressing very low levels of antigen, bypassing ineffective host immune responses. Furthermore, the modularity of the platform allows for engineering of TCRs that effectively target viral variants. In this review, we discuss the progress made in the development of ImmTAV molecules as therapeutics for functional cure of chronic hepatitis B and HIV, from concept to the clinic.

1. Introduction

Modern antiretroviral therapy (ART) provides durable control of HIV, with restoration of health and a normal lifespan, thanks to the development of safe, tolerable and convenient treatment regimens. However, ART is a lifelong commitment; virological relapse occurs if therapy is interrupted, due to the presence of long-lived cells harbouring stably integrated HIV proviruses. These viral reservoirs are primarily in lymphoid tissue and are established within the first few days of infection. While the recent approval of long-acting antiretrovirals may usher in a new era for HIV management, offering people living with HIV (PLWH) a reprieve from daily medication, they are not yet suitable for or accessible to all and do not eliminate the need for indefinite treatment.[1]

The ultimate goal of cure research is to achieve durable suppression of HIV with a finite course of therapy that is safe, acceptable, scalable and accessible. The three cases of complete cure through haematopoietic stem cell transplant (HSTC), Timothy Ray Brown, Adam Castillejo and now the 'New York patient', have galvanised the field.[2–4] The key to success in these individuals was the elimination of HIV reservoirs through myeloablative interventions, coupled with engraftment of allogeneic HIV-resistant donor cells. However, these individuals all required HSTC for treatment of life-threatening cancers; the risks of the

procedure preclude its use outside of a clinical indication.[5] A functional cure or remission, on the other hand, may be feasible without such risks. Here, the aim is to achieve at minimum, durable suppression of viral load below the transmission threshold, with a low relapse rate, for at least 2 years; an optimum scenario would be elimination of replication-competent proviruses, which are the source of rebound viremia.[6]

Natural immune responses to HIV, particularly CD8^{+} T cells, play a key role in containing the virus and may even clear reservoirs in rare individuals.[7] Furthermore, non-human primate (NHP) studies have shown that induction of virus-specific CD8^{+} T cells by vaccination could confer control of viremia following challenge with pathogenic strains of simian immunodeficiency virus (SIV).[8] Considerable effort has therefore been devoted to designing therapeutic vaccine strategies to elicit similarly potent T cell responses against HIV. However, promising results in NHP have proved challenging to replicate in humans. This indicates that multiple facets of the CD8^{+} T cell response are important, including specificity, sensitivity to antigenic stimulus, antiviral functionality and access to sites of virus production.

Other immunotherapeutic approaches that are currently being investigated for HIV include broadly neutralising antibodies, adoptive cell and gene therapies including chimeric antigen receptor (CAR) T

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cells, Toll-like receptor agonists and immune checkpoint inhibitors.[9] These have the potential to overcome some of the hurdles encountered with therapeutic vaccines, although to date no single agent has shown durable impact on virological relapse after withdrawal of ART. The consensus is, therefore, that a combination strategy will be needed. The aforementioned interventions have been recently reviewed elsewhere and are beyond the scope of this review.[10] It is also important to note that while there is considerable overlap in the concepts of CAR and T cell receptor (TCR)-transduced T cells, the key differences between them reflect their distinct modes of antigen recognition. Here, we will focus on TCR-adapted strategies.

2. Evolution of adoptive T cell therapy for HIV

The rationale for adoptive T cell therapies directed against HIV lies in observations made over three decades that CD8^{+} T cell responses exert a degree of control in most individuals, which was evident in the pre-ART era in the highly variable lag time from initial infection to AIDS, ranging from months to years. This underpinned attempts to enhance HIV-specific T cell responses, initially by expanding autologous HIV-specific T cell clones *ex vivo* prior to re-infusion into patients, without success.[11] Administration in patients with uncontrolled viremia resulted in rapid viral escape and poor persistence of the transferred T cells. As a first step towards the generation of more potent T cell products with the capacity to overcome viral escape, phage display was used to isolate and enhance the affinity of a natural TCR from an HIV-positive individual with a strong response to an HLA-A*02:01-restricted Gag p17 epitope. CD8^{+} T cells transduced with the affinity-enhanced TCR were able to control replication of diverse HIV isolates *in vitro*, through recognition of the cognate epitope and known escape variants.[12] TCR-transduced T cells with either wild-type (natural) or enhanced affinity were produced for a phase I trial in PLWH; however, two patients received infusions with wild-type TCR-transduced T cells only and the results were not reported (NCT00991224).

Improvements in ART regimens, together with the successful application of virus-specific T cell therapies in the setting of post-transplant viral infections provided impetus to re-visit the approach. *Ex vivo* expanded HIV-specific cytolytic T cell (CTL) clones (HXTC) are an adaptation of virus-specific T cell therapy that has been used successfully for treatment of post-transplant viral infections such as EBV, CMV and adenovirus.[13] HXTC are expanded from peripheral blood lymphocytes by stimulation with pools of peptides spanning conserved regions within the structural and regulatory proteins of HIV. They were shown to eliminate latently infected CD4^{+} T cells from ART-treated individuals *ex vivo*.[14,15] HXTC have been generated under Good Manufacturing Practice (GMP) conditions and tested in 6 ART-treated individuals in a proof-of-concept study. Infusions were well tolerated and autologous CD8^{+} T cells sampled post-infusion had demonstrable antiviral activity *ex vivo*.[15,16] However, no significant reduction in viral reservoirs was observed, which was attributed to the absence of a latency-reversing agent (LRA). Latency reversal is considered necessary to drive viral antigen expression and thus sensitise reservoirs to lysis by the infused HXTC. A combination strategy involving administration of an LRA, vorinostat, followed by HXTC infusions is currently being evaluated in a Phase I trial (NCT03212989). To improve the coverage of viral variants that may be archived in HIV reservoirs, the HXTC platform was modified to incorporate mosaic antigens as the stimulus, resulting in multi-antigen-specific polyclonal T cells against non-escaped epitopes (HST-NEETs). A phase I trial evaluating HST-NEETs in ART-treated individuals has been initiated (NCT03485963). The scalability of these HIV-specific T cell products may be improved by using allogeneic or naïve donor cells. However, as with HXTCs they may need to be combined with LRAs to ensure that there is sufficient antigenic stimulus for their activation.

3. Innovations to develop modular TCR-based bispecific agents

Despite progress towards the generation of 'off-the-shelf' T cell therapies, clinical application remains challenging due to the complexities of manufacture and dosing, the potential for alloreactivity and on occasions, the need for lymphodepletion. By contrast, antibodies are not donor-restricted and can be rapidly produced at scale. However, the range of antigenic targets for antibodies is limited to cell surface proteins, which account for approximately 10% of cellular proteins, and they may be sequestered by cell-free viral proteins if these are secreted in large quantities. In the case of HIV, only Env glycoproteins are expressed on the infected cell surface; Env proteins are highly prone to mutation, exquisitely adapted to evade antibody neutralisation and are also secreted as non-functional forms that act as an immunological decoy. These limitations also apply to antibody-based bispecifics such as dual affinity retargeting agents (DARTs). While the HLA restriction of TCRs limits their application, a key advantage over antibodies is that the repertoire of antigen targets includes the vast majority of cellular proteins, including those expressed intracellularly, provided that they are processed into short peptides for presentation by HLA molecules on the cell surface. The most highly conserved regions of the HIV proteome are found primarily in Gag and Pol proteins, which are expressed intracellularly. TCR-based therapies therefore offer the potential to target functionally conserved viral proteins, which are less likely to acquire mutations due to the detrimental impacts these have on viral fitness, as also indicated by structure-based network analysis.[17,18]

TCRs are heterodimeric membrane-spanning proteins with affinities for cognate peptide-HLA (pHLA) typically in the micromolar range. They display inherent cross-reactivity, enabling them to sample millions of potential targets through brief interactions.[19,20] The majority (~95%) of TCRs consist of an α and β chain, each comprised of a variable and constant domain. The α and β chain variable domains in turn each consist of three complementarity-determining region (CDR) loops that interact with the target pHLA. Transformation of membrane-bound TCRs into soluble molecules with high specificity for pHLA was dependent on several feats of protein engineering. Truncation of the transmembrane domains, which was necessary to solubilise the TCR, rendered the αβ heterodimer unstable. This was overcome by the introduction of a novel non-native disulphide bridge between the two constant domains of the TCR (Fig. 1).[21] For further details on solubilization of TCRs we refer the readers to the following recent review.[22]

To engineer a natural, membrane bound TCR (1) into a soluble, bispecific TCR (4) the transmembrane domain is removed, and a disulphide bond added to stabilize the TCR (2). The CDRs of the TCR are affinity enhanced using technologies such as phage display (3). An effector domain is fused to the TCR to provide bispecificity (4).

The first soluble TCRs to be developed were targeted to cancer antigens. Cancer-specific TCRs were isolated from naïve TCR libraries and affinity-enhanced by up to a million-fold (picomolar range) to enable targeting of peptides that are expressed at such low levels on malignant cells that they normally escape immune surveillance. This was achieved through systematic introduction of successive mutations in the CDRs, coupled with screening against a panel of pHLAs to ensure selection of TCRs with a high degree of specificity for cognate pHLA and rejection of any potentially cross-reactive TCRs.[23–25] Finally, to convert TCRs into therapeutic proteins, an antibody (single chain variable fragment, scFv) specific for CD3 was fused to the TCR β chain, generating a bispecific molecule. When the soluble TCR is bound to pHLA on the surface of a cancer cell the anti-CD3 scFv redirects neighbouring T cells, irrespective of their specificity, to form an immune synapse. This results in activation of the redirected T cell, triggering the release of pro-inflammatory cytokines including IFNγ, TNFα, IL-2 and IL-6, and the discharge of lytic molecules that destroy the cancer cell.[26,27] This approach was successfully applied to the discovery of TCRs specific for cancer testis antigens, enabling the development of the first Immune mobilising monoclonal T cell receptors Against Cancer (ImmTAC) molecules.[24] The ImmTAC
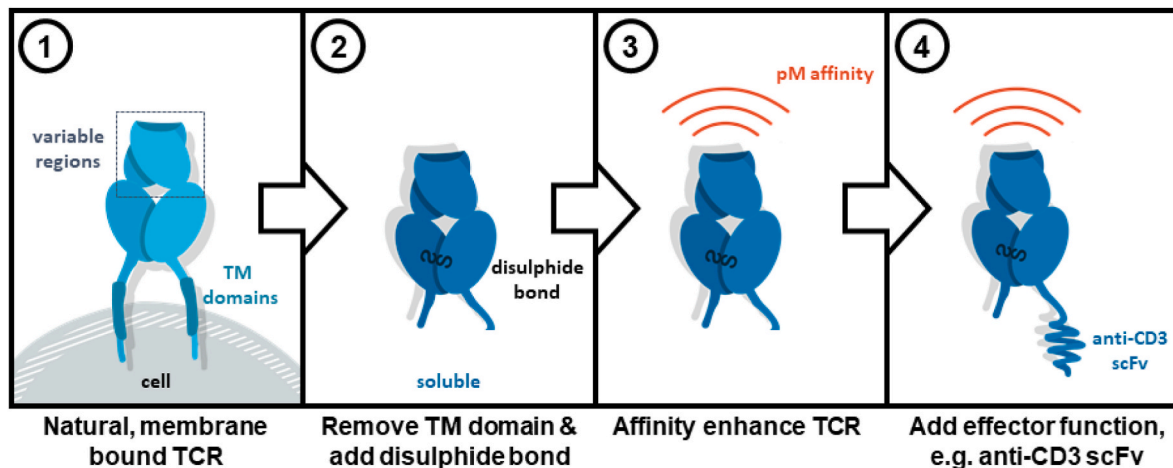
Fig. 1. Transformation of a natural TCR into a soluble TCR bispecific retargeting molecule.

mechanism of action is illustrated in Fig. 2. Further mechanistic studies have revealed that ImmTAC molecules can redirect and activate not only CD8^{+} but also CD4^{+} T cells, eliciting the release of the aforementioned pro-inflammatory cytokines as well as chemokines including macrophage inflammatory protein-1α/β.[24,27,28]

A cancer cell presents tumour-derived peptide on HLA class I molecules (1). ImmTAC with a high affinity TCR specific for the peptide-HLA complex binds and recruits a nearby T cell, regardless of its specificity, via binding of the anti-CD3 scFv (2). The T cell forms an immune synapse with the cancer cell, leading to T cell activation. The activated T cell releases pro-inflammatory cytokines and perforin, which forms pores in the cancer cell membrane, allowing cytolytic granules to enter (3). The cytolytic granules trigger apoptosis of the cancer cell (4).

The ImmTAC tebentafusp (KIMMTRAK®) is the world's first approved TCR therapeutic, developed for the treatment of metastatic uveal melanoma, an aggressive disease with a median survival from symptom onset of approximately 1 year.[29] Tebentafusp consists of an affinity-enhanced TCR-*anti*-CD3 bispecific targeting an HLA-A*02:01-restricted peptide from the gp100 antigen, which is expressed at high levels in melanoma. In a pivotal Phase III randomised controlled trial, HLA-A*02:01–positive patients with metastatic uveal melanoma were allocated to either tebentafusp monotherapy or investigators' choice of immune checkpoint inhibitor. The tebentafusp arm showed significantly longer overall survival.[30] This trial was not only the first to demonstrate clinical efficacy with a TCR therapeutic but also the first to show successful treatment of a solid tumour with a soluble bispecific agent. Consistent with the mechanism of action, therapeutic dosing was associated with cytokine release syndrome (CRS), however, in the vast majority of patients CRS severity was graded I/II and symptoms rapidly reversed with standard supportive therapy and/or corticosteroid. Furthermore, of the minority who required corticosteroids after the first dose of tebentafusp (26%), only one day's treatment was needed in nearly half of these (10%).[30,31] Several ImmTAC molecules targeting other cancer antigens have since been developed and are currently under clinical evaluation.[32]

4. Application of TCR-adapted therapies to viral infections

The success of tebentafusp has shown that a soluble TCR-bispecific can overcome several challenges in cancer immunotherapy, including penetration into solid tumours and activity in the context of low target antigen expression and in an immunosuppressive microenvironment. Chronic viral infections present similar challenges to cancers, reflecting adaptations to evade immune surveillance or to subvert host immune responses.[33] These include the persistence of replication-competent viral genomes in latent or intermittently transcribing states, enabling infected cells to avoid detection by CTLs, the accumulation of genetic mutations in CD8^{+} T cell epitopes that may impair recognition, and effector T cell exhaustion, manifested by upregulation of co-inhibitory receptors.[34] Furthermore, in the case of HIV, replication-competent proviruses persist in rare, long-lived CD4^{+} T cell clones with a central memory phenotype, which may be selected for survival through immune-editing.[35] These similarities lend support for exploring the potential to use TCR-bispecifics to eliminate virus-infected cells (Fig. 3).

ImmTAX molecules have been developed for oncology applications (ImmTAC) and chronic viral infections (ImmTAV). The ImmTAC recognizes tumour-derived peptides presented on HLA, while the ImmTAV recognizes HLA-presented viral peptides. In both cases, the anti-CD3 scFv redirects polyclonal T cells to the target cells resulting in cell lysis. *Tebentafusp (uveal melanoma) was licensed by the FDA in 2022; other ImmTAC molecules are in phase I/II trials.

4.1. Hepatitis B virus

Chronic hepatitis B (CHB) affects over 250 million people globally and causes significant morbidity and mortality, primarily due to the complications of end-stage liver disease and hepatocellular carcinoma (HCC).[36,37] The risk of serious disease can be reduced by long-term treatment with antivirals but is not completely eliminated, therefore, there is a high unmet need for a functional cure. Hepatitis B virus (HBV) establishes a reservoir in hepatocytes, episomally as covalently closed circular DNA (cccDNA) which is the source of infectious virus, and also
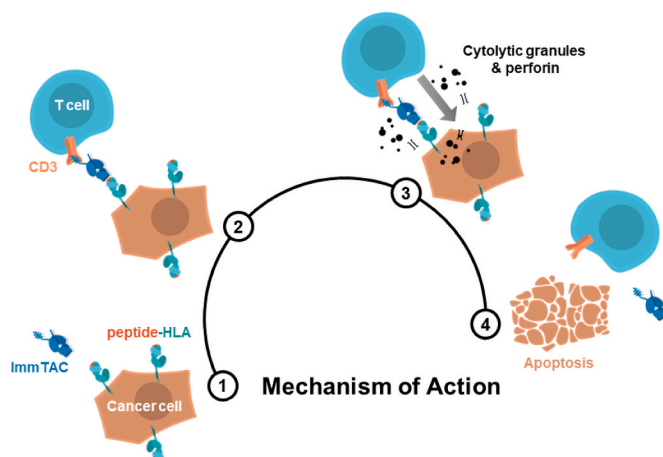

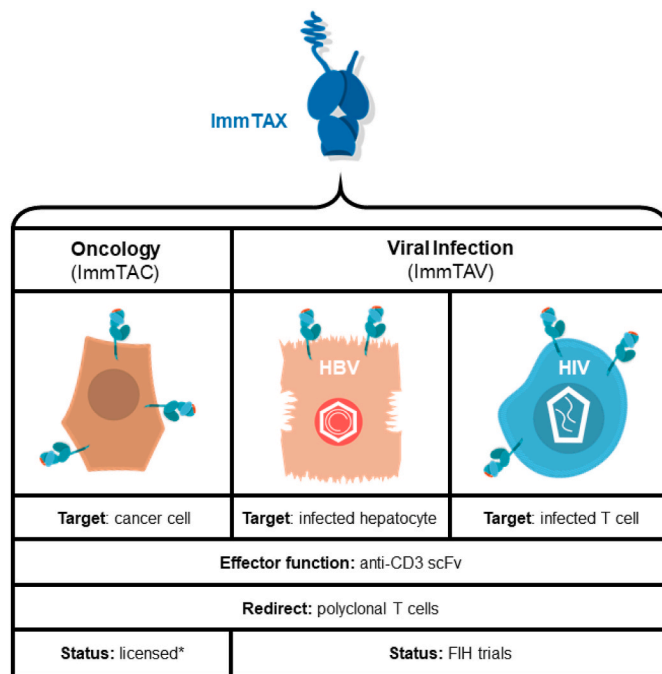**Fig. 2.** Mechanism of action of ImmTAC molecules.

Fig. 3. Immune mobilising monoclonal T cell receptors against X disease (ImmTAX®).

via integration of HBV DNA into host chromosomes. Both forms of viral DNA serve as transcriptional templates for hepatitis B surface antigen (HBsAg).[38] Continuous secretion of HBsAg is thought to promote an immunosuppressive or immune tolerant environment in the liver, which leads to exhaustion of HBV-specific T cells.[39,40] Spontaneous loss of HBsAg occurs infrequently in treatment-naïve adults or after prolonged treatment with antiviral therapy.[41,42] Functional cure, defined as sustained HBsAg loss, with or without HBsAb seroconversion, is considered a realistic goal given these observations. Various interventions to eliminate, or permanently silence HBV DNA are being actively pursued, including approaches to reactivate exhausted HBV-specific T cells.[43] TCR-adapted therapies, conversely, do not depend on restoration of these responses but engage functional non-HBV-specific T cells instead.

The LioCyx platform developed by Lion TCR uses messenger RNA (mRNA)-encoded HBV-specific TCRs to redirect T cells against HBV peptide. The mRNA is delivered by electroporation into *ex vivo* activated autologous T cells, which are then infused back into the patient. The short half-life of the TCRs enables safer dosing than with conventional adoptive T cell therapies, which typically persist *in vivo* for several weeks.[44] Because immune-mediated clearance of HBV, whether spontaneous or iatrogenic, is associated with liver inflammation and hepatocyte death ('liver flares') the risks and benefits of using T cell therapies in CHB are finely balanced.[45] Application of TCR-based therapies to HBV-driven HCC, a cancer for which there is substantial unmet need, has shown that risks associated with on-target activity in the liver can be managed without compromising clinical efficacy. In a phase 1 dose escalation study, 8 patients with advanced HBV-associated HCC received repeated infusions of TCR-transduced T cells that recognise an HBV peptide expressed in HCC tissue. Three patients experienced tumour shrinkage or disease stabilisation with or without a reduction in HBV pre-genomic RNA.[46] Of note, these individuals experienced liver enzyme elevations in tandem with clinical responses, together with increases in serum chemokines and peripheral blood T cell activation. Although one individual developed increased ALT and bilirubin of grade 3 severity, these changes resolved within 12 weeks without treatment and no subject experienced serious treatment-related adverse events.[47] These data show that a TCR-directed T cell therapy can provide clinical benefit, with predictable immune-mediated liver signals that are

nevertheless tolerable and self-limiting, even in patients with advanced CHB disease. This study therefore provides a strong rationale for exploring the potential for TCR-adapted approaches to deliver a functional cure in CHB.

4.2. Soluble TCR-bispecific agents for HBV

We have adapted the ImmTAC platform (re-named Immune mobilising monoclonal TCRs Against X disease, ImmTAX) to develop the first soluble TCR-bispecific for HBV. This Immune mobilising monoclonal T cell receptor Against Viruses (ImmTAV) molecule was engineered to recognise a highly conserved HBsAg (Env)-derived HLA-A*02:01-restricted peptide with picomolar affinity. The HBV ImmTAV was able to redirect polyclonal T cells from both HBV-naïve and CHB donors towards HBsAg-positive cell lines, resulting in target cell lysis at low picomolar ImmTAV concentrations. Using sorted effector cell subsets, we also showed that the ImmTAV efficiently redirected not only CD8^{+} T cells but also CD4^{+} T cells and mucosal-associated invariant T (MAIT) cells, the latter being highly enriched in intrahepatic lymphocytes.[48] Furthermore, in an *in vitro* infection model, the ImmTAV molecule mediated specific elimination of HBV-infected targets. Importantly from a safety perspective, cytokine release due to on-target activity against HBsAg-positive cell lines could be inhibited by a corticosteroid, while the affinity enhancement of the HBV-specific TCR did not introduce cross-reactivity to peptide mimetics in uninfected primary human hepatocytes or to cellular components of whole blood from naïve donors.[26] Taken together, these studies provided preclinical proof of concept for HBV ImmTAV-mediated redirection of polyclonal T cells as a strategy to clear HBV-positive hepatocytes.

The lead HBV-specific ImmTAV molecule, IMC-I109V, has advanced to a first-in-human clinical trial, which will evaluate the safety, tolerability and antiviral effects of IMC-I109V in HLA-A*02:01-positive adults with CHB (EudraCT #2019-004212-64). HLA-A*02:01 is the most prevalent HLA allele globally, with frequencies in Asia, one of the highest burden regions, ranging from 10 to 34% (http://www.allelefrequencies.net). To date a single dose of 0.8 mcg, which was based on the minimum anticipated biological effect level, has been well tolerated and not associated with CRS or any adverse event (n=3).[50]

4.3. Soluble TCR-bispecific agents for HIV

Building on the work of Varela-Rohena et al. we have used the ImmTAX platform to develop soluble TCR-bispecific molecules against HIV peptides, initially targeting the same HLA-A*02:01-restricted Gag p17 epitope (Gag$_{77-85}$).[12] The first ImmTAV molecules were engineered to maintain the 'polyspecificity', or capacity to recognise viral variants, of the parental TCR (Fig. 4). We showed that HIV-specific ImmTAV molecules could redirect polyclonal CD8^{+} T cells from HIV-naïve donors towards HIV-infected CD4^{+} T cells *in vitro*, leading to specific killing at picomolar drug concentrations. Effector memory and terminally differentiated effector CD8^{+} T cell subsets were more efficient killers than central memory or naïve subsets, likely reflecting differences in lytic granule content.[28,51] *Ex vivo* polyclonal CD8^{+} T cells from ART-treated PLWH were also capable of eliminating autologous CD4^{+} T cells when redirected by the ImmTAV; in these studies, patients' CD4^{+} T cells were stimulated *in vitro* to reactivate latent HIV prior to co-culture with autologous CD8^{+} T cells and latency reversal was demonstrated by intracellular staining for Gag protein expression. ImmTAV redirection led to a greater reduction in Gag-positive cells than the patients' pre-existing HIV-specific CTL responses alone, highlighting the capacity of ImmTAV molecules to bypass exhausted virus-specific T cell responses[52] (Fig. 4).

Sequence diversity in archived HIV proviruses renders them insensitive to natural HIV-specific CTL. This may be overcome by engineering TCRs that recognise the variants of interest or by combining multiple ImmTAV molecules targeting different epitopes (1). ImmTAV molecules
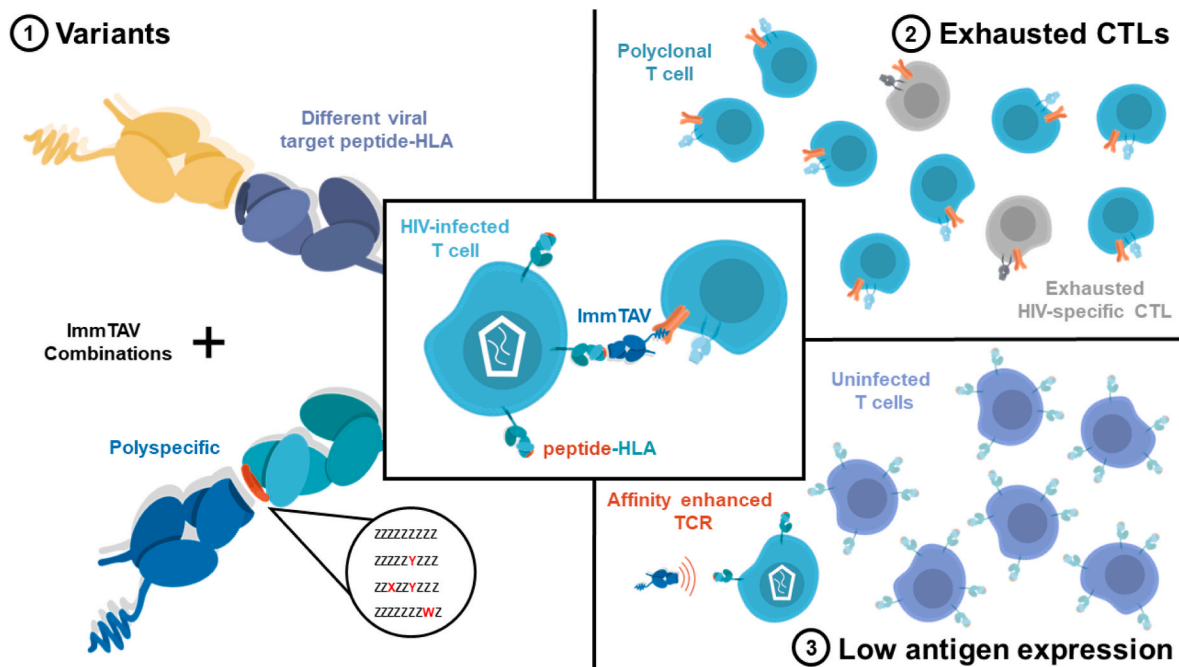
Fig. 4. ImmTAV molecules aim to overcome the failure of natural HIV-specific CD8$^+$ T cells to eliminate HIV reservoirs.

bypass exhausted HIV-specific CTL by redirecting polyclonal T cells (2). ImmTAV molecules are engineered to bind target pHLA with high affinity, enabling them to detect and eliminate infected cells even at low epitope densities (3).

The premise of using LRAs to reactivate latent HIV and thus sensitise infected CD4$^+$ cells to CTLs has dominated HIV cure research for over a decade. However, the results of clinical trials testing LRAs such as histone deacetylase inhibitors, the most widely explored drug class to date, have been disappointing.[53–55] Nevertheless, recent data indicate that HIV reservoirs may be transcriptionally active in the first few years of ART, with Gag p24 protein being detected in gut-associated and peripheral lymphoid tissues.[56–58] This opens up the possibility of targeting these antigen-positive, non-productively infected cells using ImmTAV molecules, without the need for LRAs. The plausibility of this approach may depend on the viral epitope density on the infected cells. Using labelled affinity-enhanced TCRs specific for Gag$_{77-85}$ we have shown that productively infected CD4$^+$ T cells express approximately 10–50 epitopes per cell.[52] Further studies with TCRs specific for other viral epitopes may help to determine the susceptibility of reservoirs to ImmTAV-mediated killing as a function of pHLA levels.

The 'prototype' HIV ImmTAV molecules described here underwent modifications to produce the candidate drug, IMC-M113V, which has advanced to a first-in-human trial. This dose-escalation study will evaluate the safety, pharmacokinetics and antiviral activity of IMC-M113V in HLA-A*02:01-positive ART-treated PLWH (EudraCT #2021-002008-11). HLA-A*02:01 prevalence ranges from approximately 23% in Southern Africa, the most affected region globally, to 45% in Western Europe.

5. Future directions

The ImmTAX platform has focused primarily on HLA-A*02-presented peptides to date, as HLA-A*02 alleles represent the most common subgroup of HLA class I alleles across the world. Nevertheless, ImmTAX restricted by other HLA class I alleles have been successfully developed to address ethnic variation in allele frequencies in the patient populations of interest. However, as HLA restriction is a barrier to universal application, donor-unrestricted targeting via non-classical class I molecules such as HLA-E is an attractive possibility. HLA-E has very limited polymorphism, with only two alleles in humans. While its primary function is to regulate NK cell cytotoxicity by presenting signal peptides from classical class I molecules, this pathway is exploited by several pathogens to enable immune evasion.[59,60] For example, HIV Nef protein downregulates HLA-A and -B molecules but does not affect HLA-E expression, protecting infected cells from lysis by both CD8$^+$ T cells and NK cells. A macaque homologue of HLA-E, Mamu-E, was found to play a central role in vaccine-mediated protection of rhesus macaques against infectious SIV challenge, via the induction of broad Mamu-E restricted SIV-specific CD8$^+$ T cells.[61,62] These encouraging results triggered a search for T cell responses to HIV peptides presented by HLA-E.[63,64] However, successful development of a TCR-adapted therapy will depend on the identification of peptides that can form a stable complex with HLA-E. The most well characterised HIV peptide, Gag$_{275-283}$ shows very low affinity for HLA-E in its native form, which raises questions regarding whether this peptide is presented *in vivo* at sufficient levels to be recognised by CD8$^+$ T cells.[65]

A further consideration for the development of soluble TCRs against HIV is the need to ensure coverage of viral variants that may be archived in cellular reservoirs. Greater diversity is seen in PLWH who initiate ART during the chronic phase than in acute infection. As has been elegantly demonstrated in clinical studies with broadly neutralising antibodies (bNAbs), in which the duration of post-treatment control was determined by the sensitivity of viral reservoirs to the infused antibodies, pre-existing escape mutations could potentially confer resistance to TCR-adapted therapies.[66] This can be mitigated in several ways: (1) targeting of highly conserved viral epitopes, in which mutation is constrained by fitness costs, (2) engineering polyspecificity (tolerability to escape mutations without cross-reactivity to irrelevant antigens, as discussed earlier), and (3) combination strategies. The latter could comprise a combination of two or more ImmTAV molecules with different specificities and/or combining with a complementary targeting domain such as a bNAb or a DART. Finally, engineering of multispecific molecules could provide advantages over combinations by reducing complexity in manufacturing and clinical dosing.[67,68] The modularity of the ImmTAX platform is exemplified by a recent study that showed how an alternative effector function, a PD-1 agonist, can be incorporated for application to autoimmune disease, paving the way for new formats of TCR therapeutics.[69]

Declaration of competing interest

Acknowledgements

References

1 Thornhill J, Orkin C. Long-acting injectable HIV therapies: the next frontier: Republication. *Curr Opin HIV AIDS*. 2021;16:98–105. https://doi.org/10.1097/coh.0000000000000670.

2 Allers K, Hütter G, Hofmann J, et al. Evidence for the cure of HIV infection by CCR5Δ32/Δ32 stem cell transplantation. *Blood*. 2011;117:2791–2799. https://doi.org/10.1182/blood-2010-09-309591.

3 Gupta RK, Peppa D, Hill AL, et al. Evidence for HIV-1 cure after CCR5Δ32/Δ32 allogeneic haemopoietic stem-cell transplantation 30 months post analytical treatment interruption: a case report. *Lancet HIV*. 2020;7:e340–e347. https://doi.org/10.1016/s2352-3018(20)30069-2.

4 Hsu J, Besien KV, Glesby MJ, et al. HIV-1 remission with CCR5Δ32Δ32 haplo-cord transplant in a U.S. woman:IMPAACT P1107 [CROI ABSTRACT 65]. In special issue: abstracts from the 2022 Conference on Retroviruses and Opportunistic Infections. *Top Antiv Med*. 2022;30:23.

5 Verheyen J, Esser S, Kordelas L. More on shift of HIV tropism in stem-cell transplantation with CCR5 delta32/delta32 mutation. *N Engl J Med*. 2014;371: 2437–2438. https://doi.org/10.1056/nejmc1412279.

6 Lewin SR, Attoye T, Bansbach C, et al. 2019 W. Group. Multi-stakeholder consensus on a target product profile for an HIV cure. *Lancet HIV*. 2020;8:e42–e50. https://doi.org/10.1016/s2352-3018(20)30234-4.

7 Li JZ, Blankson JN. How elite controllers and posttreatment controllers inform our search for an HIV-1 cure. *J Clin Invest*. 2021;131. https://doi.org/10.1172/jci149414.

8 Stephenson KE, Li H, Walker BD, Michael NL, Barouch DH. Gag-specific cellular immunity determines in vitro viral inhibition and in vivo virologic control following simian immunodeficiency virus challenges of vaccinated rhesus monkeys. *J Virol*. 2012;86:9583. https://doi.org/10.1128/jvi.00996-12. –9.

9 Barr L, Jefferys R. A landscape analysis of HIV cure-related clinical research in 2019. *J Virus Erad*. 2020;6, 100010. https://doi.org/10.1016/j.jve.2020.100010.

10 W.G. 1 (Understanding H. reservoirs), Z. Ndhlovu, N. Chomont, Z. Brumme, K. Deng, L. Jasenosky, R. Jefferys, A. Orta-Resendiz, W.G. 2 (HIV reservoir measurement), F. Mardarelli, M. Nijhuis, K. Bar, B. Howell, A. Schneider, G. Turk, R. Nabatanzi, W.G. 3 (Mechanisms of virus control), J. Blankson, J.V. Garcia, M. Paiardini, J. van Lunzen, C. Antoniadi, F.H. Côrtes, W.G. 4 (Targeting the provirus), S. Valente, O.S. Søgaard, R.S. Diaz, M. Ott, R. Dunham, S. Schwarze, S.P. Patrigeon, J. Nabukenya, W.G. 5 (Targeting the immune system), M. Caskey, B. Mothe, F.S. Wang, S. Fidler, D. SenGupta, S. Dressler, M. Matoga, W.G. 6 (Cell and gene therapy), H.-P. Kiem, P. Tebas, C. Kityo, B. Dropulic, M. Louella, K.T. Das, W.G. 7 (Paediatric remission and cure), D. Persaud, A. Chahroudi, K. Luzuriaga, T. Puthanakit, J. Safrit, G. Masheto, W.G. 8: (Social cure) behavioral and ethical aspects of, K. Dubé, J. Power, J. Salzwedel, U. Likhitwonnnawut, J. Taylor, O.L. Nuh, K. Dong, E.N. Kankaka Deeks SG, Archin N, Cannon P, Collins S, Jones RB, de Jong MAWP, Lambotte O, Lamplough R, Ndung'u T, Sugarman J, Tiemessen CT, Vandekerckhove L, Lewin SR, Ias TIAS(, , G.S.S. working group, C.L. Group, Deeks S, Lewin S, de Jong M. Research priorities for an HIV cure: international AIDS society global scientific strategy 2021. *Nat Med*. 2021;27:2085–2098. https://doi.org/10.1038/s41591-021-01590-5.

11 Koenig S, Conley AJ, Brewah YA, et al. Transfer of HIV-1-specific cytotoxic T lymphocytes to an AIDS patient leads to selection for mutant HIV variants and subsequent disease progression. *Nat Med*. 1995;1:330–336. https://doi.org/10.1038/nm0495-330.

12 Varela-Rohena A, Molloy PE, Dunn SM, et al. Control of HIV-1 immune escape by CD8 T cells expressing enhanced T-cell receptor. *Nat Med*. 2008;14:1390–1395. https://doi.org/10.1038/nm.1779.

13 Lee P-H, Keller MD, Hanley PJ, Bollard CM. Virus-specific T cell therapies for HIV: lessons learned from hematopoietic stem cell transplantation. *Front Cell Infect Microbiol*. 2020;10:298. https://doi.org/10.3389/fcimb.2020.00298.

14 Lam S, Sung J, Cruz C, et al. Broadly-specific cytotoxic T cells targeting multiple HIV antigens are expanded from HIV+ patients: implications for immunotherapy. *Mol Ther*. 2015;23:387–395. https://doi.org/10.1038/mt.2014.207.

15 Sung JA, Patel S, Clohosey ML, et al. HIV-specific, ex vivo expanded T cell therapy: feasibility, safety, and efficacy in ART-suppressed HIV-infected individuals. *Mol Ther*. 2018;26:2496–2506. https://doi.org/10.1016/j.ymthe.2018.08.015.

16 Sung JA, Lam S, Garrido C, et al. Expanded cytotoxic T-cell lymphocytes target the latent HIV reservoir. *J Infect Dis*. 2015;212:258–263. https://doi.org/10.1093/infdis/jiv022.

17 Rihn SJ, Wilson SJ, Loman NJ, et al. Extreme genetic fragility of the HIV-1 capsid. *PLoS Pathog*. 2013;9, e1003461. https://doi.org/10.1371/journal.ppat.1003461.

18 Gaiha GD, Rossin EJ, Urbach J, et al. Structural topology defines protective CD8+ T cell epitopes in the HIV proteome. *Science*. 2019;364:480–484. https://doi.org/10.1126/science.aav5095.

19 Rudolph MG, Stanfield RL, Wilson IA. HOW tcrs bind mhcs, peptides, and coreceptors. *Annu Rev Immunol*. 2006;24:419–466. https://doi.org/10.1146/annurev.immunol.23.021704.115658.

20 Sewell AK. Why must T cells be cross-reactive? *Nat Rev Immunol*. 2012;12:669–677. https://doi.org/10.1038/nri3279.

21 Boulter JM, Glick M, Todorov PT, et al. Stable, soluble T-cell receptor molecules for crystallization and therapeutics. *Protein Eng Des Sel*. 2003;16:707–711. https://doi.org/10.1093/protein/gzg087.

22 Robinson RA, McMurran C, McCully ML, Cole DK. Engineering soluble T-cell receptors for therapy. *FEBS J*. 2021;288:6159–6173. https://doi.org/10.1111/febs.15780.

23 Li Y, Moysey R, Molloy PE, et al. Directed evolution of human T-cell receptors with picomolar affinities by phage display. *Nat Biotechnol*. 2005;23:349–354. https://doi.org/10.1038/nbt1070.

24 Liddy N, Bossi G, Adams KJ, et al. Monoclonal TCR-redirected tumor cell killing. *Nat Med*. 2012;18:980–987. https://doi.org/10.1038/nm.2764.

25 Dunn SM, Rizkallah PJ, Baston E, et al. Directed evolution of human T cell receptor CDR2 residues by phage display dramatically enhances affinity for cognate peptide-MHC without increasing apparent cross-reactivity. *Protein Sci*. 2006;15:710–721. https://doi.org/10.1110/ps.051936406.

26 Fergusson JR, Wallace Z, Connolly MM, et al. Immune-Mobilizing monoclonal T cell receptors mediate specific and rapid elimination of hepatitis B–infected cells. *Hepatology*. 2020;72:1528–1540. https://doi.org/10.1002/hep.31503.

27 Middleton MR, McAlpine C, Woodcock VK, et al. Tebentafusp, A TCR/anti-CD3 bispecific fusion protein targeting gp100, potently activated antitumor immune responses in patients with metastatic melanoma. *Clin Cancer Res*. 2020;26: 5869–5878. https://doi.org/10.1158/1078-0432.ccr-20-1247.

28 Boudousquie C, Bossi G, Hurst JM, Rygiel KA, Jakobsen BK, Hassan NJ. Polyfunctional response by ImmTAC (IMCgp100) redirected CD8+ and CD4+ T cells. *Immunology*. 2017;152:425–438. https://doi.org/10.1111/imm.12779.

29 Rantala ES, Hernberg M, Kivelä TT. Overall survival after treatment for metastatic uveal melanoma: a systematic review and meta-analysis. *Melanoma Res*. 2019;29: 561–568. https://doi.org/10.1097/cmr.0000000000000575.

30 Nathan P, Hassel JC, Rutkowski P, et al. Imc.-202 investigators, overall survival benefit with tebentafusp in metastatic uveal melanoma. *N Engl J Med*. 2021;385: 1196–1206. https://doi.org/10.1056/nejmoa2103485.

31 Ikeguchi A, Sacco JJ, Luke JJ, et al. Analysis of the effect of systemic corticosteroids on survival from tebentafusp in a phase 3 trial of metastatic uveal melanoma. In: *Proceedings of the American Society of Clinical Oncology Annual Meeting, Chicago, IL USA*. 2022. Poster 9584.

32 Immunocore, Pipeline. Growing a pipeline with potential global reach (accessed March 24, 2022) https://www.immunocore.com/our-science/pipeline.

33 Vance RE, Eichberg MJ, Portnoy DA, Raulet DH. Listening to each other: infectious disease and cancer immunology. *Sci Immunol*. 2017;2. https://doi.org/10.1126/sciimmunol.aai9339.

34 Paiardini M, Dhodapkar K, Harper J, Deeks SG, Ahmed R. Editorial: HIV and cancer immunotherapy: similar challenges and converging approaches. *Front Immunol*. 2020;11:519. https://doi.org/10.3389/fimmu.2020.00519.

35 Huang S-H, McCann CD, Mota TM, Wang C, Lipkin SM, Jones RB. Have cells harboring the HIV reservoir been immunoedited? *Front Immunol*. 2019;10:1842. https://doi.org/10.3389/fimmu.2019.01842.

36 Akinyemiju T, Abera S, Ahmed M, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level. *JAMA Oncol*. 2017;3:1683–1691. https://doi.org/10.1001/jamaoncol.2017.3055.

37 Iannacone M, Guidotti LG. Immunobiology and pathogenesis of hepatitis B virus infection. *Nat Rev Immunol*. 2021;22:19–32. https://doi.org/10.1038/s41577-021-00549-4.

38 Wooddell CI, Yuen M-F, Chan HL-Y, et al. RNAi-based treatment of chronically infected patients and chimpanzees reveals that integrated hepatitis B virus DNA is a source of HBsAg. *Sci Transl Med*. 2017;9:eaan0241. https://doi.org/10.1126/scitranslmed.aan0241.

39 Tsukuda S, Watashi K. Hepatitis B virus biology and life cycle. *Antivir Res*. 2020;182, 104925. https://doi.org/10.1016/j.antiviral.2020.104925.

40 Hu J, Protzer U, Siddiqui A. Revisiting hepatitis B virus: challenges of curative therapies. *J Virol*. 2019;93. https://doi.org/10.1128/jvi.01032-19.

41 Zhou K, Contag C, Whitaker E, Terrault N. Spontaneous loss of surface antigen among adults living with chronic hepatitis B virus infection: a systematic review and pooled meta-analyses. *Lancet Gastroenterology Hepatology*. 2019;4:227–238. https://doi.org/10.1016/s2468-1253(18)30308-x.

42 Lok AS, Zoulim F, Dusheiko G, et al. Durability of hepatitis B surface antigen loss with nucleotide analogue and peginterferon therapy in patients with chronic hepatitis B. *Hepatology Commun*. 2019;4:8–20. https://doi.org/10.1002/hep4.1436.

43 Gane E, Verdon DJ, Brooks AE, et al. Anti-PD-1 blockade with nivolumab with and without therapeutic vaccination for virally suppressed chronic hepatitis B: a pilot study. *J Hepatol*. 2019;71:900–907. https://doi.org/10.1016/j.jhep.2019.06.028.

44 Tan AT, Yang N, Krishnamoorthy TL, et al. Use of expression profiles of HBV-DNA integrated into genomes of hepatocellular carcinoma cells to select T cells for immunotherapy. *Gastroenterology*. 2019;156:1862–1876. https://doi.org/10.1053/j.gastro.2019.01.251. e9.

45 Ghany MG, Feld JJ, Chang K-M, et al. Serum alanine aminotransferase flares in chronic hepatitis B infection: the good and the bad. *Lancet Gastroenterology Hepatology*. 2020;5:406–417. https://doi.org/10.1016/s2468-1253(19)30344-9.

46 Tan AT, Yang N, Krishnamoorthy TL, et al. Use of expression profiles of HBV-DNA integrated into genomes of hepatocellular carcinoma cells to select T cells for immunotherapy. *Gastroenterology.* 2019;156:1862–1876. https://doi.org/10.1053/j.gastro.2019.01.251. e9.

47 Meng F, Zhao J, Tan AT, et al. Immunotherapy of HBV-related advanced hepatocellular carcinoma with short-term HBV-specific TCR expressed T cells: results of dose escalation, phase I trial. *Hepatol Int.* 2021;15:1402–1412. https://doi.org/10.1007/s12072-021-10250-2.

48 Tang X-Z, Jo J, Tan AT, et al. IL-7 licenses activation of human liver intrasinusoidal mucosal-associated invariant T cells. *J Immunol.* 2013;190:3142–3152. https://doi.org/10.4049/jimmunol.1203218.

50 Bourgeois S, Lim Y-S, Gane EJ, et al. IMC-I109V, a novel T cell receptor (TCR) bispecific (ENVxCD3) designed to eliminate HBV-infected hepatocytes in chronic HBV patients: initial data from a first-in-human study (SAT437). In: *The International Liver Congress.* EASL); 2022:S872–S873.

51 Wolint P, Betts MR, Koup RA, Oxenius A. Immediate cytotoxicity but not degranulation distinguishes effector and memory subsets of CD8+ T cells. *J Exp Med.* 2004;199:925–936. https://doi.org/10.1084/jem.20031799.

52 Yang H, Buisson S, Bossi G, et al. Elimination of latently HIV-infected cells from antiretroviral therapy-suppressed subjects by engineered immune-mobilizing T-cell receptors. *Mol Ther.* 2016;24:1913–1925. https://doi.org/10.1038/mt.2016.114.

53 McMahon DK, Zheng L, Cyktor JC, et al. A phase 1/2 randomized, placebo-controlled trial of romidespin in persons with HIV-1 on suppressive antiretroviral therapy. *J Infect Dis.* 2021;224:648–656. https://doi.org/10.1093/infdis/jiaa777.

54 Rasmussen TA, Tolstrup M, Brinkmann CR, et al. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. *Lancet Hiv.* 2014;1: e13–e21. https://doi.org/10.1016/s2352-3018(14)70014-1.

55 Fidler S, Stöhr W, Pace M, et al. Antiretroviral therapy alone versus antiretroviral therapy with a kick and kill approach, on measures of the HIV reservoir in participants with recent HIV infection (the RIVER trial): a phase 2, randomised trial. *Lancet.* 2020;395:888–898. https://doi.org/10.1016/s0140-6736(19)32990-3.

56 Wu G, Zuck P, Goh SL, et al. Gag p24 is a marker of human immunodeficiency virus expression in tissues and correlates with immune response. *J Infect Dis.* 2021;224: 1593–1598. https://doi.org/10.1093/infdis/jiab121.

57 Baxter AE, Niessl J, Fromentin R, et al. Single-cell characterization of viral translation-competent reservoirs in HIV-infected individuals. *Cell Host Microbe.* 2016; 20:368–380. https://doi.org/10.1016/j.chom.2016.07.015.

58 Graf EH, Pace MJ, Peterson BA, et al. Gag-positive reservoir cells are susceptible to HIV-specific cytotoxic T lymphocyte mediated clearance in vitro and can Be detected in vivo. *PLoS One.* 2013;8, e71879. https://doi.org/10.1371/journal.pone.0071879.

59 Strong RK, Holmes MA, Li P, Braun L, Lee N, Geraghty DE. HLA-E allelic variants correlating differential expression, peptide affinities, crystal structures, and thermal stabilities. *J Biol Chem.* 2003;278:5082–5090. https://doi.org/10.1074/jbc.m208268200.

60 Grimsley C, Ober C. Population genetic studies of HLA-E evidence for selection. *Hum Immunol.* 1997;52:33–40. https://doi.org/10.1016/s0198-8859(96)00241-8.

61 Hansen SG, Wu HL, Burwitz BJ, et al. Broadly targeted CD8+ T cell responses restricted by major histocompatibility complex E. *Science.* 2016;351:714–720. https://doi.org/10.1126/science.aac9475.

62 Malouli D, Hansen SG, Hancock MH, et al. Cytomegaloviral determinants of CD8 + T cell programming and RhCMV/SIV vaccine efficacy. *Sci Immunol.* 2021;6. https://doi.org/10.1126/sciimmunol.abg5413.

63 Yang H, Rei M, Brackenridge S, et al. HLA-E–restricted, Gag-specific CD8+ T cells can suppress HIV-1 infection, offering vaccine opportunities. *Sci Immunol.* 2021;6, eabg1703. https://doi.org/10.1126/sciimmunol.abg1703.

64 Bansal A, Gehre MN, Qin K, et al. HLA-E–restricted HIV-1–specific CD8+ T cell responses in natural infection. *J Clin Invest.* 2021;131. https://doi.org/10.1172/jci148979.

65 Barber C, Souza VAD, Paterson RL, et al. Structure-guided stabilization of pathogen-derived peptide-HLA-E complexes using non-natural amino acids conserves native TCR recognition. *Eur J Immunol.* 2022. https://doi.org/10.1002/eji.202149745.

66 Mendoza P, Gruell H, Nogueira L, et al. Combination therapy with anti-HIV-1 antibodies maintains viral suppression. *Nature.* 2018;561:479–484. https://doi.org/10.1038/s41586-018-0531-2.

67 Xu L, Pegu A, Rao E, et al. Trispecific broadly neutralizing HIV antibodies mediate potent SHIV protection in macaques. *Science.* 2017;358:85–90. https://doi.org/10.1126/science.aan8630.

68 Elshiaty M, Schindler H, Christopoulos P. Principles and current clinical landscape of multispecific antibodies against cancer. *Int J Mol Sci.* 2021;22:5632. https://doi.org/10.3390/ijms22115632.

69 Curnock AP, Bossi G, Kumaran J, et al. Cell-targeted PD-1 agonists that mimic PD-L1 are potent T cell inhibitors. *JCI Insight.* 2021;6. https://doi.org/10.1172/jci.insight.152468.