ERAP1: A potential therapeutic target for a myriad of diseases

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**Abstract**

**Introduction:** Endoplasmic Reticulum Aminopeptidase 1 (ERAP1) is a key regulator of the peptide repertoire displayed by Major Histocompatibility Complex I (MHC I) to circulating CD8+ T cells and NK cells. Studies have highlighted the essential requirement for the generation of stable peptide MHC I in regulating both innate and adaptive immune responses in health and disease.

**Areas covered:** Here, we review the role of ERAP1 in peptide trimming of N-terminally extended precursors that enter the ER, before loading on to MHC I, and the consequence of loss or downregulation of this activity. Polymorphisms in ERAP1 form multiple combinations (allotypes) within the population, and we discuss the contribution of this ERAP1 variation, and expression, on disease pathogenesis, including the resulting effect on both innate and adaptive immunity. We consider the current efforts to design inhibitors based on approaches using rational design and small molecule screening, and the potential effect of pharmacological modulation on the treatment of autoimmunity and cancer.

**Expert Opinion:** ERAP1 is fundamental for the regulation of immune responses, through generation of the presented peptide repertoire at the cell surface. Modulation of ERAP1 function, through design of inhibitors, may serve as a vital tool for changing immune responses in disease.

**Keywords**: ERAP1, antigen processing and presentation, MHC I, CD8+ T cells, NK cells, cancer, autoimmunity.

**Article Highlights:**

* Endoplasmic Reticulum Aminopeptidase 1 (ERAP1) is a key regulator of peptide repertoire displayed at the cell surface, by trimming N-terminally extended peptides to the optimal length (8-10amino acids) for MHC I binding and presentation.
* Alterations in ERAP1 activity significantly alter the quality and quantity of the peptide repertoire, which has the ability to induce robust T cell or NK cell responses.
* ERAP1 is highly polymorphic within the population, and polymorphisms affect the function of ERAP1 activity.
* ERAP1 and ERAP2 polymorphic variants are associated with autoimmune conditions and cancer, and has been shown to destroy immunogenic peptides associated with melanoma and murine colorectal carcinoma.
* Modulation of ERAP1, either by siRNA or pharmacological inhibition, has shown significant alterations in peptide repertoire, as well as tumour rejection in lymphoma and colorectal carcinoma models.

**1. Introduction**

**1.1 MHC I immune recognition**

A fundamental component of host immunity relies on the presentation of endogenously derived peptides at the cell surface by Major Histocompatibility Complex class I (MHC I) molecules. These peptide MHC I (pMHC I) complexes are presented on the surface of nucleated cells for interaction with Natural Killer (NK) cells as part of the innate immune response, and surveillance and recognition by CD8+ cytotoxic T lymphocytes in adaptive immunity. NK cells provide the first immune defense against viral infection and tumour cell development through the expression of activating and inhibiting receptors. pMHC I act as ligands for a subset of these receptors, for example, killer immunoglobulin receptors (KIR) which deliver inhibitory signals upon engagement of self-MHC I. Alterations in pMHC I, including changes in peptide presentation or a reduction in expression levels, result in a loss of the KIR engagement and subsequent loss of inhibitory signals, promoting NK-mediated lysis [1]. Conversely, CD8+ T cells monitor pMHC I expressed on cells and interaction between the T cell receptor (TCR) and pMHC I is crucial for development, tolerance and activation of T cells. TCR recognition of pMHC I that display foreign or ‘non-self’ peptides, arising from viral infection or malignant transformation, promotes cell lysis eliminating the infected or transformed cell.

The assembly of pMHC I for stable cell surface presentation is therefore fundamental in regulating these innate and adaptive immune responses to altered cells. A crucial component of pMHC I assembly is the generation of optimal peptide ligands for presentation and subsequent recognition, which ultimately controls immune responses; aberrant peptide generation can prevent the appropriate immune response in cancer, or conversely, an immune response towards healthy cells resulting in self attack and autoimmunity [2-4].

**1.2 Antigen Processing and Presentation**

The MHC I antigen processing and presentation pathway is formed by a number of important steps performed by molecules and chaperones that results in the production of stable peptide loaded MHC I molecules that are able to egress to the cell surface for presentation to CD8+ T cells and NK cells (figure 1).

The initial steps in antigen presentation begin in the cytosol, where the first processing event is undertaken by the proteasome, or under inflammatory conditions, the immunoproteasome. Proteins are targeted for degradation by the ubiquitin-proteasome system, and results in the generation of smaller peptide fragments [5]. The proteasome/immunoproteasome cleavage pattern often results in a hydrophobic C-terminal residue, which is optimal for loading into the F-pocket of the peptide binding groove of most MHC I [5]. A subset of proteasomal products are translocated into the ER by the transporter associated with antigen processing (TAP) heterodimer. Interestingly, the preferences of TAP for longer peptides (10-16 amino acids) means that many peptides enter the ER as extended precursors that will require further processing by ER aminopeptidase 1 (ERAP1), and to a lesser extent ERAP2, before they are of optimal length for MHC I binding [6]. pMHC I assemble in the ER from a polypeptide heavy chain, 2microglobulin and peptide, usually of 8-10 amino acids in length. These peptides are edited and loaded on to MHC I in association with the peptide loading complex (PLC); TAP, tapasin, ERp57 and calreticulin (figure 1). Interestingly, the peptide binding groove of most MHC I is highly polymorphic, giving rise to multiple MHC I alleles that can each display a variety of different peptides. Binding of peptides into the peptide binding groove stabilizes the pMHC I complex, which subsequently dissociates from the PLC and transits to the cell surface for immunosurveillance [4].

**1.3 ERAP1 regulates immune responses through peptide trimming**

The peptide trimming function of ERAP1 has been demonstrated by multiple groups and confirmed in a number of cell lines and animal models, and is responsible for the primary peptide processing event in the ER [7-12]. ERAP1, ERAP2 and insulin-regulated aminopeptidase (IRAP), an enzyme expressed in endosomes with analogous peptide trimming function with ERAP1/2, are members of the M1 zinc metalloproteases, specifically the oxytocinase subfamily, all of which are characterized by the presence of two key motifs in the active site; GXMEN substrate binding site and HEXXH-X18E zinc binding motif [13-15]. ERAP2 and IRAP share 49% and 43% sequence homology with ERAP1, mostly within the conserved active site domains. Interestingly, mice only express ERAP1 (ERAAP in mice) and IRAP, with no ERAP2 gene.

Like other antigen processing machinery components, ERAP1 is upregulated in response to IFN-, and tissue distribution of expression follows MHC I expression [10-12]. ERAP1 trims N-terminally extended precursors to their final length of 8-9amino acids, and has been demonstrated to trim both free peptides and those bound to MHC I, which may act to ‘protect’ optimal ligands from ERAP1 activity [16-21].

Cell surface expression of MHC I depends on both the quality and quantity of the peptide supply in the ER and are affected by the presence of ERAP1 activity. Several groups have observed a reduced number of pMHC I complexes presented at the cell surface in the absence of ERAP1 activity. This reduction is largely dependent upon the MHC I alleles expressed and has been shown to vary accordingly, but studies have reported up to 70% reduction in mice and 30% in humans, highlighting the requirement for ERAP1 in generating the optimal peptide cargo [7,22-24]. This was further highlighted in ERAP1 knock-out mice that revealed the reduction of pMHC I surface expression was a result of a faster dissociation of the complexes at the cell surface, rather than a slower pMHC I assembly in the ER [7]. These pMHC I expressed in the absence of ERAP1 are therefore less stable and have a shorter half-life when compared to those generated in presence of ERAP1 activity [7,22,24].

Although MHC I alleles play a significant role in the repertoire of peptide presented at the cell surface (immunopeptidome), ERAP1 is an additional peptide editor that influences the repertoire displayed. Indeed, the quality and quantity of peptides at the cell surface is affected by the presence of ERAP1 activity [7,22,25]. In the absence of ERAP1, the array of peptides presented by splenocytes are significantly different and the pMHC complexes unstable and structurally unique [22]. Additionally, loss of ERAP1 activity resulted in fewer peptides being presented and novel peptides identified were longer in length, with a higher abundance of N-terminal extensions [7,22,25,26]. Furthermore, Chen et al confirmed this increase in N-terminal extended peptides in human cell lines, but somewhat surprisingly, also reported an increase in the number of C-terminal extended epitopes in response to silencing of ERAP1, likely a result of the position 2 arginine anchor for HLA-B\*27 binding and lack of destruction of these epitopes in the absence of ERAP1 [27]. Interestingly, not all peptides entering the ER require ERAP1 activity for presentation, with a subset of peptides remaining unaffected by the loss of ERAP1 function [7]. In peptidomes derived from ERAP1 KO mice, it was revealed that many of the conventional pMHC I complexes seen in wild-type (WT) cells were lost in place of alternative complexes that were more immunogenic, demonstrated by the induction of a robust T cell responses to ERAP1KO cells in WT mice, as well as an increase in the number and immunogenicity of peptides associated with non-classical MHC I [7,22,25,28]. Several studies have also indicated an important function of ERAP1 in the destruction of epitopes by over-trimming, effectively removing a pool of peptides from the antigenic repertoire [7,25,29,30]. Thus, in the absence of ERAP1 over-trimming, these peptides are presented at the cell surface, changing the immune landscape.

As a regulator of the peptide repertoire, ERAP1 plays a significant role in T cell responses to viral epitopes, establishing and altering immunodominance hierarchy patterns in Lymphocytic Choriomeningitis Virus (LCMV), murine Cytomegalovirus (mCMV) and vaccinia infection [25,26]. Furthermore, ERAP1 can modulate innate immune responses such as NK cell lysis, and the loss of ERAP1 affects the susceptibility of tumours to NK mediated killing [31,32]. During the initial stages of pathogen recognition, ERAP1 knock-out mice display an increase in activation of NK and NKT cells, an increase in IFN- production as well as an increase in the phagocytic activity of splenic DCs and macrophages [33].

ERAP1 is understood to be the dominant aminopeptidase in the ER for trimming N-terminally extended precursors, however ERAP2 is also thought to be important in this role[34]. Sharing 49% sequencing homology to ERAP1, ERAP2 has distinctly different trimming preferences compared to ERAP1 [34,35]. ERAP1 displays a hierarchy of trimming for naturally occurring amino acids, and preferentially trims peptides with N-terminal extensions that contain hydrophobic amino acids, such as leucine and valine, whereas more polar charged amino acids, such as arginine, are preferentially trimmed by ERAP2 [8,21,34,36]. Interestingly, like other aminopeptidases, ERAP1 and ERAP2 are unable to trim X-Pro bonds, and alongside the inability of TAP to transport peptides with X-Pro motif, results in the transport of peptides into the ER with N-terminal extensions requiring ERAP trimming [6,11]. This suggests the requirement of these aminopeptidases for the generation of peptides where proline at p2 is an anchor residue for the HLA allele binding groove, such as H2-Ld [37], HLA-B\*7, HLA-B\*35 and HLA-C\*04. ERAP1 and ERAP2 are able to form heterodimers in cells resulting in an improved efficiency to generate peptides such as the HIV derived peptide, GPGRAFVTI, from extended precursors, suggesting that some peptides may require both ERAP1 and ERAP2 activity for their presentation [34]. The exact role of ERAP2 in antigen processing still remains largely unclear given that 25% of individuals lack any detectable ERAP2 expression due to the presence of a single nucleotide polymorphism (SNP; rs2248374) which targets the protein for degradation [38].

**2. Potential of ERAP1 as a therapeutic target**

**2.1 ERAP1 structure**Recently, crystallographic analysis has improved our understanding of both peptide specificity and the trimming mechanism of ERAP1, and to a lesser extent ERAP2 [39-41]. ERAP1 is characterised as a four-domain structure that undergoes a conformational change from open to closed, reconfiguring the active site region during this transition. This conformational change is also observed with other M1 aminopeptidases, including IRAP [42]. The four domains are structured as follows; domain I caps off the N-terminal side of the active site region, domain II contains the active site and catalytic domains, domain III is a smaller domain thought to be responsible for the hinge action to facilitate conformational changes, and domain IV forms a concave surface which is thought to contain a regulatory domain that binds the C-terminus of the substrate [40,41,43,44].

The open conformation of ERAP1 is perceived to be peptide receptive, and upon binding substrate, closes around the peptide to form the closed, enzymatically active structure. When investigating the structural basis for catalytic activity, the open conformation revealed the highly conserved catalytic Tyr438 residue, to be facing away from the Zn atom, as well as containing a poorly structured S1 pocket, essential for determining substrate specificity [36,40,41]. This analysis suggests that ERAP1 may cycle between different conformations in order to i) bind substrate, ii) cleave the peptide and iii) release the product. In addition, in the closed conformation, a large internal cavity is formed, likely to accommodate large peptides of up to 16 amino acids in length, which further validates findings from in vitro analyses which suggests optimal ERAP1 activity towards peptides <16 amino acids in length [16]. More recently, Giastas et al reported two high-resolution crystal structures (1.68A and 1.72A) with bound peptide analogs, revealing peptides are trapped within the internal cavity in the closed conformation, despite differences in peptide length (10 vs. 15 amino acids) [45].

Based on structural data alongside biochemical analysis, the proposed regulatory region, which is distinct from the active site, is thought to encourage longer peptide trimming by binding to their C-terminus, promoting the adoption of a closed conformation and increasing catalytic activity [41,43,44]. Interestingly, a substrate of 15 amino acids was shown to be bound in the internal cavity at both C- and N-terminal regions, with interactions in the C-terminal binding site between residues Tyr684, Lys685 and Arg807 and the C-terminal residue of the peptide influencing the enzymatic activity in a substrate dependent manner [45].

Further analysis is required to elucidate the exact contribution of this regulatory site for the catalytic activity of ERAP1 towards peptides of different lengths and sequences. This knowledge is essential for the design of inhibitors that reduce enzymatic activity, as currently most approaches for inhibitor design have been focussed on the active site in the closed conformation [46]. Indeed, targeting the C-terminal binding area may be the key to enabling ERAP1-specific inhibition, as this region is distinct in sequence and structure from ERAP2 and IRAP [47,48].

**2.2 Trimming mechanisms of ERAP1**

A distinct hallmark of ERAP1 trimming activity is the ability to efficiently trim peptides into their final length for MHC I loading. Although the exact mechanism for ERAP1 length preferences is not clear, structural studies, biochemical analysis and cellular assays have contributed to two distinct hypotheses for the trimming mechanism. The first is based on evidence from crystal structures and in vitro biochemical evidence that suggests ERAP1 itself acts as a molecular ruler, trimming peptides in solution and using its enzymatic properties and structural composition to optimise peptides to the correct length for MHC I, ceasing in activity at <8 amino acids (see above) [12,16,18,19,41]. Conversely, ERAP1 has been shown to trim peptides to the correct length only in the presence of the relevant peptide binding MHC I allele [9]. Generation of the cognate peptide epitope was only observed in the presence of both ERAP1 and H2-Ld. Absence of H2-Ld in the assay led to the destruction of peptide ligand by ERAP1. Furthermore, we and others have demonstrated the ability of ERAP1 to trim N-terminally extended peptides whilst tethered to the MHC I [7,17,20,21,49]. Nevertheless, the competition between ERAP1 trimming activity and stable binding of peptides to MHC I is the major driving force for the optimisation of peptide cargo in either scenario. It is highly plausible that ERAP1 can undertake both mechanisms of peptide trimming in different circumstances and stimuli, such as during inflammatory events, working at different stages of the pathway. Further investigation is important to determine these characteristics and the exact mechanisms of trimming function.

**2.3 Contribution of ERAP1 to disease pathogenesis**

ERAP1 and ERAP2 are prominent risk factors for MHC I associated autoimmune and autoinflammatory diseases, as well as hypertension, viral infection and cancer (reviewed in [2,50]). In autoimmune conditions, the epistasis of ERAP1 with specific HLA alleles, HLA-B\*27, HLA-B\*51, HLA-C\*06:02 and HLA-A\*29 in Ankylosing Spondylitis (AS), Behçet’s Disease (BD), Psoriasis and Birdshot Chorioretinopathy (BC) respectively, demonstrates a strong genetic influence [50]. Many of these disease associations are linked to the expression of natural polymorphic variants of ERAP1 and ERAP2 and will be discussed in more detail below. These associations suggest peptide handling and the generation of peptides by ERAP1 influences susceptibility to HLA associated diseases, and that MHC I bound peptides are highly relevant in their pathogenesis. Many studies to date have focussed on the link between ERAP1 polymorphism, HLA allele expression and autoimmune conditions, and efforts have centred around elucidation of the pathogenic mechanisms, in particular in AS. These studies have been extensively reviewed in [2,3,50-52].

Expression of ERAP1, as well as efficient enzymatic activity is also important in cancer, and an effective immune evasion strategy by tumours is the downregulation of MHC I expression [53]. This lack of cell surface presentation may be due to targeting MHC I itself, or more frequently, targeting aspects of the antigen processing pathway resulting in the reduction/loss of MHC I. Alterations in the protein expression of ERAP1 in multiple tumours of different histological origins has been documented; including leukaemia and lymphoma, melanoma and cervical, lung, colon, prostate, kidney and bladder carcinomas [54-59] (and the role of ERAP1 in cancer has been reviewed in [2]). Importantly, those tumours that revealed a low level of ERAP1 and/or ERAP2 expression correlated with a lower enzymatic activity [56]. The comparison between normal and malignant tissues from the same individual suggests the alterations observed in ERAP1 expression occur during the transformation process. Furthermore, the increase in expression of ERAP1 and ERAP2 by transfection in HeLa cells that were categorised as ERAP-low, resulted in an upregulation of MHC I expression, suggesting that defective expression in ERAP1 and ERAP2 has a direct effect on abnormal MHC I levels [55]. The most common phenotype observed in tumour tissue was low/down-regulation in expression of ERAP1 and ERAP2 compared to normal tissue, such as ovarian, breast, lung cancer as well as in melanoma and an aggressive form of neuroblastoma [54,56,57]. However, in colon and thyroid cancer, an upregulation of ERAP1 expression was observed when compared to the low expression in normal tissue [56]. This observation is consistent with the discovery that expression of ERAP1 destroys the cross-protective tumour antigen, GSW11, in the CT26 murine colon carcinoma model [29], and suggests the increase in ERAP1 expression in certain types of cancer is an immune evasion tactic used to increase the destruction of potentially immunogenic tumour antigens.

The role of ERAP1 in infection has not been studied as extensively; however, ERAP1 was shown to be essential for eliciting protective immunity to the parasite Toxoplasma gondii in mice [60]. Furthermore, in LCMV, mCMV and vaccinia infection, an increase in the length of the viral peptides was observed in the absence of ERAP1. When measuring the frequency of CD8+ T cell specific for viral epitopes, a lack of antigen processing by ERAP1 profoundly altered the responses to LCMV, mCMV and vaccinia viral derived peptides, suggesting a significant role in establishing the hierarchy of immunodominance in viral infection [25,26]. Draenert et al revealed immune selection pressure causes an amino acid change in an HLA-B\*57 HIV immunodominant epitope that results in altered antigen processing by ERAP1. The mutation, A146P, in the HIV-gag protein prevents N-terminal trimming of the peptide precursor by ERAP1, leading to a reduction in CD8+ T cell responses [61]. In a more recent study, we highlight the effect of ERAP1 trimming function on the generation of hepatitis C immunodominant viral epitopes, with a poor ERAP1 activity unable to sufficiently generate a 9 amino acid HLA-B\*27 restricted epitope required for sufficient CD8+ T cell response and viral clearance [62].

The contribution and effect of ERAP1 activity in disease may be different depending on the disease type due to discordant levels of expression observed in different tumour types, as well as allotypic variation affecting function [21,56]. Nevertheless, in cancer and viral infections, failure of ERAP1 to produce tumour or viral antigens, through the lack of trimming activity or destruction of epitopes, results in altered immunodominance hierarchies and can also lead to the failure to induce an effective immune response, as observed in LCMV and mCMV infection and the susceptibility of the tumour-associated epitopes GSW11 and MART-1 in colorectal carcinoma and melanoma to over-processing by ERAP1 [25,26,29,30]. The role of ERAP1 in autoimmunity, however, results in an over-active immune response, most likely due to the alterations in the peptide repertoire presented on specific HLA alleles. In addition to the obvious potential effect on the generation of specific auto-antigenic epitopes, of which only a minority have been identified and their relationship with ERAP1 is currently unknown [63,64], it may be that the change in the state of the global peptide repertoire presented on disease associated HLA alleles influences potential innate and adaptive immune responses, through interaction with KIR on NK cells or CD8+ T cells. Given the broad nature of ERAP1 disease association, and the significance of the resulting innate and adaptive immune responses, ERAP1 may serve as a unique therapeutic target applicable for multiple disease treatments.

**2.4 Polymorphic variation in ERAP1**

ERAP1 is polymorphic within the population, but to a lesser degree than the variation observed in MHC I alleles. Genome wide association studies have identified common ERAP1 SNPs associated with a number of diseases; rs26653 R127P (associated with AS, psoriasis and HPV-positive cervical carcinoma survival), rs2287987 M349V (associated with AS), rs30187 K528R (associated with AS, type 1 diabetes, psoriasis, multiple sclerosis, hypertension and inflammatory bowel disease), rs10050860 D575N (associated with AS), rs17482078 R725Q (associated with AS) and rs27044 Q730E (associated with AS, psoriasis and HPV-positive cervical carcinoma survival) [50,65-68]. We and others have demonstrated the presence of multiple SNP combinations in the ERAP1 gene, forming haplotypes that encode multiple distinct ERAP1 protein variants, termed ‘allotypes’, that affect the processing function [21,23,69]. Indeed, one of the most prevalent ERAP1 variants in the Caucasian population contains all six common disease associated SNPs (\*001, Hap10, 26.2% compared to 13.7% WT) [21,23,69]. Studies have investigated the contribution of single and combinations of SNPs on the enzymatic function of ERAP1, using both in vitro and in cellulo assays in an attempt to elucidate the contribution of these SNPs and their relevance in disease. The most widely studied SNP effect on ERAP1 function is rs30187, encoding K528R, and has been linked with an increased risk of hypertension and associated with autoimmune disease [50,68]. The observed reduction in function of K528R mutant is probably related to impaired inter-domain interactions, as this SNP is present in the ‘hinge’ region of domain III, in a position likely to affect transition in conformation [21,41,70]. Interestingly, other SNPs within ERAP1 are located near the active site region (rs27895 - G346D and M349V) or within the proposed C-terminal peptide binding cavity (R725Q and Q730E) [40,41]. Q730E affects the enzymatic activity of ERAP1 in a substrate specific manner, shown by a 30% increase in efficiency for trimming a polar/charged precursor of SIINFEKL (LEQLEK-SIINFEKL) compared with a relatively hydrophobic N-terminal extension (AIVMK-SIINFEKL) [18,71]. Therefore, Q730E is likely to influence activity dependent on the substrate length and amino acid properties due to the involvement of this residue on substrate C-terminus binding. Most notable is the fact that the activity of an ERAP1 allotype may not be accurately predicted as a sum of the individual SNPs that are present, as shown by the combined effect of residues at positions 528 and 575 on the altered processing of HLA-B\*27 ligands, as well as our data highlighting ERAP1 \*001 (Hap10) containing 6 SNPs as hypoactive, even though this variant contains R528/Q725 and Q725/E730 combinations that in individual allotypes were shown to be hyperactive [21,72]. The diverse alterations that each SNP confers on activity, as well as the possible combination of effect, give rise to a spectrum of enzymatic functions for each individual allotype [21]. Assessment of allotype trimming activity identified within the population gives rise to three broad categories of trimming function: efficient, hypoactive and hyperactive. However, there are caveats for each allotype, often having multiple complexities of trimming, dependent upon the sequence of the N-terminal extension, and quite possibly the peptide backbone itself [21]. ERAP1 is co-dominantly expressed, and we have identified a number of ERAP1 allotype combinations within the population. Most individuals express heterozygous ERAP1 allotypes, and the overall ERAP1 trimming activity within a cell was found to be dependent upon the function and contribution of both ERAP1 allotypes expressed, as observed in AS and HPV-induced oropharyngeal squamous cell carcinoma (OPSCC) [23,73].

Indeed, cells expressing ERAP1 consisting of disease associated SNPs present a peptide repertoire that is altered in ways that reflects the complexity of ERAP1 allotypes and the functional effect of the SNPs they possess. In the context of the AS associated Q730E SNP, the E730 variant HLA-B\*27 peptidome contains a higher abundance of shorter peptides compared to Q730[51]. In addition, a decrease in affinity and increase in peptide length in the context of the K528R variant was observed. In BD, which is associated with \*001 (Hap10) ERAP1 that has a reduced trimming capacity, the peptidome of cells expressing HLA-B\*51:08 and ERAP1 \*001 (Hap10) revealed presentation of a greater proportion of peptides that were suboptimal in length with a lower affinity [74]. Examination of HPV-induced cancer, specifically OPSCC, revealed the requirement of efficient ERAP1 activity to generate HPV-specific epitopes, and that the functional activity of ERAP1 allotypes expressed within OPSCC correlated with the level of tumour infiltrating CD8+ T cells [73]. These studies highlight the influence of ERAP1 variation on trimming function and the global peptide repertoire presented at the cell surface. Therefore, the expression of polymorphic ERAP1 allotypes results in an altered peptide repertoire, which serves to modulate immune responses; preventing those necessary for tumour rejection or inducing response to ‘self’ in autoimmunity.

**3. Validation of ERAP1 for therapeutic potential**

The ability to modulate the presented peptide repertoire, to either induce protective immune responses or reduce an auto-reactive response, could have a huge potential therapeutic benefit. To assess this, validation of ERAP1 for therapeutic potential was first indicated through enzymatic assays which revealed ERAP1 activity was significantly reduced in the presence of the broad spectrum aminopeptidase inhibitor leucinethiol (LeuSH) [9,22,29,31]. This was further reinforced by the use of siRNA which significantly reduced ERAP1 protein expression and in turn, functional enzymatic activity, in cell-based model systems. The consequence of this siRNA knock-down, or LeuSH inhibition, gave rise to changes in peptide presentation; an increase in the detection of novel peptides that are ERAP1 sensitive and destroyed in the presence of ERAP1 activity, as well as the expected reduction in the generation of known peptides that are ERAP1 dependent [9,29-31]. This effect was observed in both cell systems using an ERAP1 dependent model peptide, as well as those endogenously derived peptides requiring ERAP1 function for generation.

ERAP1 inhibition by siRNA or LeuSH in cellular systems has a varying effect on overall levels of MHC I expression at the cell surface, with initial studies reporting a down-regulation of surface MHC I in the absence of ERAP1 activity [7,22,31,32,55]. Later studies have revealed a more conflicting effect of ERAP1 down-regulation on global MHC I presentation, confirming down-regulation in some instances, but also demonstrating either no significant effect or an up-regulation in expression [32,75]. These findings indicate that the effect of ERAP1 on MHC I expression levels may be influenced by additional factors such as the specific MHC I allele (related to its peptide binding motif), the variant of polymorphic ERAP1 expressed and the cell line examined. More recently, investigations have shifted to focus on the MHC I peptide cargo presented rather than expression levels of MHC I itself. Although a significant proportion of the immunopeptidome is ERAP1 independent, changes in the global peptide repertoire presented at the cell surface are reported in the absence of ERAP1 activity [7,28,51,75]. Down-regulation of ERAP1 can lead to changes in the amino acid composition of peptides, with particular emphasis on the first amino acid, as well as an increase in the length of peptides presented. Interestingly, cells that express MHC I alleles known to present peptides with an X-Pro-Xn motif, such as HLA-B\*07 subtype, were shown to be more susceptible to alterations in ERAP1 expression/function, likely due to the requirement of ERAP1 to generate optimal ligands for these MHC I, as described above [32]. These findings may be pharmacologically significant, as being able to significantly alter the peptide repertoire presented by MHC I without abrogating overall expression levels at the cell surface would be desirable in activating CD8+ T cells in a tumour setting by avoiding MHC I down-regulation; a common immune evasion mechanism.

Further reinforcement for ERAP1 as a potential target for innate and adaptive anti-tumour immunity was demonstrated in two distinct tumour models revealing tumour growth was halted in response to modulation of ERAP1. Silencing of ERAP1 in the murine T cell lymphoma, RMA, resulted in NK mediated rejection of the lymphoma in syngeneic mice. This was due to the failure of pMHC I to engage with the Ly49C/I NK inhibitory receptors as a result of sub-optimal peptide cargo in the absence of ERAP1 [31]. In addition, we showed that a tumour specific CD8+ T cell response was elicited in ERAAP silenced CT26, resulting in tumour rejection and prolonged survival [29].

Targeting the M1 aminopeptidase family for therapeutic benefit showed initial promise of clinical effect when inhibited with the broad spectrum inhibitors, tosedostat and bestatin, proving efficacious in phase II clinical trials for treatment of AML and lung cancer [47,76,77]. Early aminopeptidase inhibitors, bestatin and amastatin, had poor potency for ERAP1 [41]. By contrast, LeuSH has a higher potency for ERAP1, but like bestatin and amastatin, it is a broad spectrum aminopeptidase inhibitor and therefore not a good candidate for pharmacological modulation of ERAP1 [47]. The subsequent association of ERAP1 with disease, the knowledge that SNPs altered ERAP1 function and greater understanding of the mechanism of action of ERAP1, led to the development of a new generation of inhibitors that were either rationally designed, or identified through small molecule screening, confirmed a greater specificity for ERAP1 inhibition [46,48,78,79]. Using solved crystal structures of ERAP1 as a guide, the rational design approach of potent peptide-based M1 aminopeptidase inhibitors using a phosphinic group as a substrate transition analog yielded the compound DG013A [46]. This compound was identified to be a potent inhibitor of ERAP1 as well as ERAP2, in the sub-nM range, and several studies have confirmed the effect of the compound on ERAP1 inhibition [46,80,81]. DG013A has been shown to enhance antigen presentation in HeLa cells stably transfected with HLA-B\*27, as well as increasing the CTL response again murine colon carcinoma CT26 [46]. In addition, this inhibitor affects innate immune responses such as the suppression of ERAP1 dependent TH17 responses in vitro and the down-regulation of macrophage phagocytosis [80,81]. Since its generation, different design approaches have produced inhibitors that have higher selectivity, but are not as potent as DG013A. Thiomersal is proposed to be an ERAP1-specific inhibitor which binds to the Zn atom in the active site, and was shown to reduce ERAP1-dependent antigen presentation in dendritic cells [79]. However, the use of this compound for pharmacological modulation may be confounded by high levels of toxicity. Exploration of the efficacy of nine weakly coordinating zinc binding groups revealed that the potency of inhibitors for ERAP1 may primarily be driven by the occupation of the active site specificity pockets [82]. In 2019, Giastas and colleagues obtained a high-resolution crystal structure (1.60A) of ERAP1 in the closed conformation, with the potent (nM) phosphinic pseudopeptide inhibitor DG046 bound [78,83]. The structures revealed DG046 to bind in the active site with a geometry that mimics a transition state analogue, and allowed detailed mapping of the internal cavity of ERAP1 in the closed conformation [83]. This was the first crystal structure solved at such high-resolution with an ERAP1 inhibitor, a major breakthrough in the barrier of structural studies and inhibitor design.

In early 2020, three small molecule compounds were identified through high throughput screening that were selective for ERAP1, but not ERAP2 and IRAP [48]. Interestingly, compounds 1 and 2 inhibited ERAP1 in in vitro L-amc fluorogenic and HeLa cellular assays, whilst compound 3, an aryl sulphonamide, activated ERAP1 L-amc activity, but inhibited in cellular assays. Interestingly, molecular docking analysis proposed compounds 1 and 2 bind in the active site region, whilst compound 3 is proposed to bind allosterically at a junction between domains II, III and IV. Furthermore, compound 3 was shown to be a more potent activator of WT ERAP1 compared to the 5-SNP ERAP1 variant differing at residues 346, 514, 528 and 730 in in vitro assays [48]. Following this interesting observation of inhibiting ERAP1 by targeting the proposed regulatory domain, a natural product originating from *Dodonaea viscosa* was identified to be a highly selective, competitive inhibitor of ERAP1 that targets the regulatory site, which usually binds the C-terminus of the peptide. These most recent developments open up a novel strategy for future inhibitor development [84]. These data suggest that modulators of ERAP1 may function as a novel tool that will aid the development of approaches to treatment of disease where immunomodulation or immunostimulation is required.

**4. Conclusions**

ERAP1 is an important aminopeptidase that functions to trim antigenic peptides to fit within the binding site of MHC I. During the last decade, the importance of this trimming function on modulation of immune responses has become clearer. Since the first identification in 2002, many studies have aimed to understand the mechanism of ERAP1, from basic trimming function to peptide preferences, the resulting effect of what peptides are presented at the cell surface, and how this modulates immune responses both under normal circumstances and in the disease context. Here we have reviewed the current body of literature surrounding ERAP1, revealing that 1) it is polymorphic within the population which gives rise to multiple allotypes with altered function, 2) both SNPs and expression of ERAP1 are associated with autoimmune conditions, cancer and viral infections, and 3) modulation of ERAP1 function, either genetically or chemically, impacts the presented peptide repertoire and influences both innate and adaptive immune responses. Taken together, these data suggest ERAP1 is likely a good therapeutic target and recent efforts by a number of key groups have focussed on the development of specific inhibitors of ERAP1 proving efficacious in modulation of function. Although there is some way to go in understanding the consequences of ERAP1 inhibition in the context of specific diseases and the HLA expressed, the effect on modulating immune responses makes it an attractive target for clinical benefit that requires further investigation.

**5. Expert Opinion – ERAP1 as a therapeutic target in disease**

Until recently, modulation of the MHC I antigen processing and presentation pathway has been an underutilised tool for clinical benefit, considering the requirement of MHC I expression to induce effective/protective T cell or NK cell responses in a myriad of diseases. Numerous components of this pathway have a significant impact the quality and quantity of MHC I expression and the displayed peptide repertoire. The dual role of ERAP1 in generating and destroying antigens suggests that any changes in ERAP1 activity will result in modifications in specific tumour/viral antigen presentation and the global peptide repertoire. The recent advances in our understanding of ERAP1-driven modulation of the peptide repertoire, and the overwhelming evidence for the impact of this on the magnitude and efficacy of immune responses, points towards the modulation of ERAP1 as being an obvious therapeutic tool for exploitation. Initial studies have used both small molecule screening and a rational design approach based on ERAP1 crystal structures to identify compounds that inhibit ERAP1 function. Although still in their infancy, these compounds have proved efficacious in their ability to inhibit ERAP1 function.

The most desirable effect of ERAP1 modulation for activation of CD8 T cells would be to alter the peptide repertoire without causing a shift in the level of MHC I expression, an immune evasion strategy well documented in tumour development, and would be applicable in cancer and viral infection. Indeed, where the modulation of responses may abrogate the presentation of ERAP1-dependent tumour/viral antigens, the upregulation of ERAP1-susceptible and novel antigens at the cell surface may mitigate this. This upregulation of alternative epitopes is likely to negate the barrier of the reduction in ERAP1-dependent epitopes due to the increased immunogenicity as observed in animal models. In the autoimmune context, inhibition of ERAP1 activity may reduce the presentation of auto-antigens, thereby preventing over-activation of immune responses. To date there have been few reports of specific auto-antigens responsible for disease pathogenesis, and the change in global peptide repertoire in cells lacking ERAP1 activity may also be key for reducing self-attack in autoimmunity through both CD8 T cell and NK cell mediated mechanisms. Indeed, autoimmune conditions represent the greatest class of diseases that are associated with ERAP1, however as yet there has been very little evidence highlighting the effect of ERAP1 modulation in these diseases, which raises questions about the efficacy of ERAP1 modulation in these conditions.

An important consideration in the modulation of ERAP1 is that despite having similar disease phenotypes, not all individuals will require the same level of ERAP1 modulation, and the level of modulation is likely to be multifactorial; expression levels of ERAP1, the allotypic variation (and resulting function) of the ERAP1, and the MHC I allele expressed in each individual. Therefore, further understanding of the relationship between ERAP1 variation and HLA allele expression is crucial in understanding the nature of altering ERAP1 function that would be beneficial in each disease context. Furthermore, recent studies have highlighted different ERAP1 allotypes exist within the population, and these allotypes can display vastly different trimming activities. As yet, investigations into small modulators of ERAP1 function have only considered these in the context of the ‘wild-type’ efficient ERAP1. Important consideration must be given to the idea that combinations of SNPs present in ERAP1 may alter the conformation of the enzyme and/or the mechanism of trimming action which may alter the level of inhibition when using a global ERAP1 inhibitor designed for ‘wild-type’. Indeed, the effect of allotypic variation on trimming function may also mean certain individuals express poor-functioning ERAP1 (in the case of \*001/Hap10) and therefore inhibition will likely not alter the activity or the peptides presented at the cell surface. Further investigation into the complexity of ERAP1 function and allotypic variation will be crucial to identify which allotypes, and therefore which individuals, will be more likely to benefit from pharmacological modulation of ERAP1.

Obvious pitfalls when developing compounds for therapeutic benefit are complications with toxicity. The most immediate concern will be involving peripheral peptidome alterations leading to ‘non-self’ recognition and an immune response directed against otherwise healthy cells in addition to those intended (e.g. tumour cells). This is likely to result in autoimmune-like features and an inflammatory response. Such problems may be mitigated by the context in which ERAP1 modulators are used, and the ability to tolerate these alterations; a short course of treatment may only be necessary to effectively boost presentation of antigens already presented in cells at a low level (e.g. GSW11). For responses to novel-self antigens, the cessation of therapy would restore original levels of antigenic peptides, removing the modulation dependent novel antigens, stopping the auto-reactive T cell responses.

Whilst the ultimate ‘Holy Grail’ would be to develop ERAP1 specific inhibitors and activators, and determine the exact mechanism of action and effect, it may not be a productive endeavour. Providing the level of toxicity is negligible, it may be more practical that compounds used are not entirely ERAP1 specific, having an effect on closely related aminopeptidases, ERAP2 and IRAP, as these are also likely to modulate the presented immunopeptidome giving rise to an altered peptide landscape which would activate CD8+ T cell responses. Important milestones in furthering our understanding of ERAP1 as a target for therapy will therefore be i) determining the specificity of the compounds, ii) understanding the change in peptide repertoire in disease specific and ERAP1 allotype specific contexts and iii) the overall level of toxicity of potential compounds, and any autoimmune-like complications. Future studies will undoubtedly build upon these findings and will need to further explore the applicability of the pharmacological benefit of altering the peptide repertoire for clinical effect, utilising ERAP1 as the essential regulator of innate and adaptive immunity through antigenic peptide processing.

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**Declaration of Interest**

E. James is on the advisory panel for Grey wolf Therapeutics. The authors have no other affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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