TEIPP antigens for T-cell based immunotherapy of immune-edited HLA class I[low] cancers

Koen A. Marijt, Elien M. Doorduijn, Thorbald van Hall[*]

Department of Medical Oncology, Leiden University Medical Center (LUMC), Leiden, The Netherlands

A B S T R A C T

T-cell based immunotherapies through checkpoint blockade or adoptive transfer are effective treatments for a wide range of cancers like melanomas and lung carcinomas that harbor a high mutational load. The HLA class I and class II (HLA-I and HLA-II) presented neoantigens arise from genetic mutations in the cancerous cells and are ideal non-self targets for the T cell-based treatments. Although some cancer patients responded with complete regression, many others are irresponsive to checkpoint blockade treatments, or relapse after initial success. One of the mechanisms by which tumors evade T cell recognition is by acquiring deficiencies in the HLA-I antigen-processing pathway, leading to downregulation of HLA-I molecules at the cell surface and thereby creating an 'invisible' tumor phenotype. Interestingly, an alternative antigen repertoire arises on these HLA-I^{low} cancer cells. We refer to this alternative antigen repertoire as TEIPP: T cell epitopes associated with impaired peptide processing. TEIPP antigens are curious non-mutated peptides from housekeeping proteins that are not presented in homeostasis. In this review, for the first time we recapitulate all our published work on TEIPP antigens, including our recent understanding of the CD8 T cell repertoire. We are convinced that TEIPP-directed T cells will be valuable resources to target immune-edited tumors that have acquired resistance to checkpoint blockade therapy.

1. Introduction

Great progress has been made in using the host's immune system to fight cancers and T-cell based immunotherapies have shown to be successful in treating patients with many different human cancers, including melanomas, lung- and renal cell carcinomas and lymphomas. Especially immune checkpoint inhibition therapies with blocking antibodies to PD-1, PDL-1, and CTLA4 proved to be effective in many patients (Chen and Mellman, 2017; Delamarre et al., 2015; Dranoff, 2013; Larkin et al., 2015). Interestingly, patients who, after an initial response, become refractory to checkpoint blockade therapy have been shown to carry tumors with mutations in the interferon-γ signal transduction pathway (Gao et al., 2016; Zaretsky et al., 2016). Inactivating mutations in the JAK1/2-STAT1 cascade effectively renders tumors 'deaf' for interferons and thereby tumor cells acquire resistance to its anti-proliferative signals. In addition, these mutations were shown to strongly hamper the capacity of tumor cells to present endogenous antigens by HLA-I molecules (Manguso et al., 2017; Sucker et al., 2017). As a result, the checkpoint-induced T cell attack becomes futile. Genetic mutations or epigenetic silencing of HLA-I molecules or components of the intracellular processing machinery, like the peptide transporter TAP, similarly result in immune-edited HLA-I[low] tumors (Garrido et al., 2010; Seliger, 2012).

Nearly a decade ago, we published a first report on a new category of tumor antigens that arises on such immune-escaped tumors and refer to them as TEIPP (van Hall et al., 2006). TEIPP-specific T cells are able to selectively recognize TAP-deficient, HLA-I[low] tumors, whereas conventional T cells were unable to do so. TEIPP antigens are non-mutated self-antigens and derive from housekeeping proteins. Since then we examined why TEIPP antigens are not presented on normal cells; how safe the exploitation of TEIPP antigens is; if TEIPP T cells are hampered by central tolerance in the thymus; if they actually exhibit similar T cell functions as conventional T cells; and the ultimate question: if TEIPP T cells can effectively be used for immune-edited cancers.

2. TEIPP as a new category of tumor antigens

Antigen-specific T cells are able to recognize peptide/MHC complexes on the surface of almost all nucleated cells. The peptides loaded into these MHC-I molecules are degradation products of intracellular proteins. Aged and misfolded proteins, or defective ribosomal products (DRIPs) are degraded by the multicatalytic proteasome in the cytosol

(Neefjes et al., 2011). The generated peptides are then translocated into the endoplasmic reticulum (ER) by the peptide transporter TAP where they have access to MHC-I in the peptide loading complex (PLC), consisting of chaperones like tapasin, ERp57 and calreticulin and trimming enzymes like ERAAP (Thomas and Tampe, 2017). Subsequently, the stabilized peptide:MHC-I complexes egress to the cell surface and can be recognized by surveilling CD8 T cells.

The first tumor antigens that were exploited for immune therapy belong to the subset of commonly expressed proteins called tumor-associated antigens (TAA). TAAs are often non-mutated antigens that are (ectopically) overexpressed in cancers. Until now most therapies have focused on TAAs like the melanoma differentiation antigens MART-1, tyrosinase and gp100. Unfortunately, most clinical trials targeting TAAs failed to show success, possibly due to central and peripheral immune tolerance mechanisms resulting in less potent T cells (Melero et al., 2014). Another category of tumor-associated antigens are cancer testis antigens (CTA). CTAs belong to a group of tumor antigens expressed on tumors and in the testis, which include MAGE-1 and NY-ESO. However, many CTAs are only expressed on a restricted number of tumor types and it is now known that presentation of CTAs is also found in low levels on healthy cells, making them less favorable to use in a therapeutic setting (Morgan et al., 2013).

Tumor-specific antigens (TSA) are a subset of highly immunogenic tumor antigens specific for transformed cancerous cells. This includes peptides derived from oncogenic viruses, like the human papilloma virus (HPV), which can cause head- and neck carcinomas or cervical neoplasms. These HPV-transformed cancer cells present virus-derived E6 and E7 peptides on the surface and therapeutic vaccination against E6 and E7 antigens has shown great successes in pre-malignant lesions (Melief et al., 2015). Another important category of TSAs are neoantigens derived from the accumulation of genetic mutations in cancer cells. Personalized designed vaccination against neoantigens showed great responses in melanoma patients (Ott et al., 2017; Sahin et al., 2017) and checkpoint therapy-induced immune responses are frequently directed towards these personal point-mutated antigens (Le et al., 2017; McGranahan et al., 2016; Snyder et al., 2014). Therefore this category of unique and tumor-specific T cell epitopes are at the forefront of scientific interest at the moment.

3. HLA class I downregulation

The vast majority of the tumor antigens described above require proteasome activity and peptide transport by TAP (Blum et al., 2013). However, most tumors eventually develop resistance and evade immune recognition (Dunn et al., 2002; Mittal et al., 2014; Sharma et al., 2017). Immunotherapy resistance can be described by 3 categories,
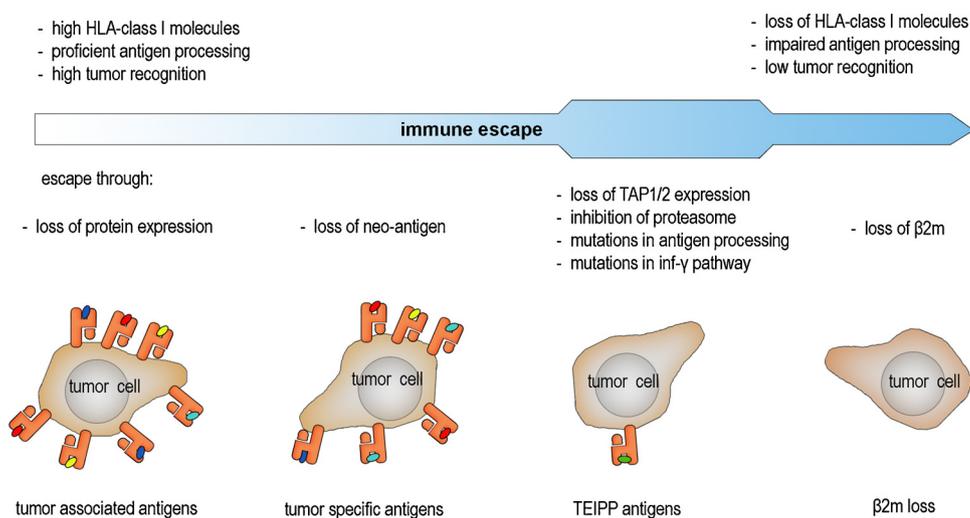
namely, "primary resistance", "adaptive resistance", and "acquired resistance" (Sharma et al., 2017). It has become clear that tumors can develop resistance against immune recognition by downregulation of HLA-I levels and thereby resist T cell immunity and checkpoint therapy (Marincola et al., 2000; Sucker et al., 2017). HLA-I down modulation in cancers is frequently found, in 50% of the primary prostate carcinomas, 43% of the primary breast carcinoma, and approximately 30% of the primary lung, colon, and cervical carcinomas (Hicklin et al., 1999). In metastatic prostate lesions, HLA-I downregulation occurred in 70% of the investigated samples, whereas 50% of the analyzed metastatic melanoma, breast, and cervical lesions had downregulation of HLA class I expression (Hicklin et al., 1999; Marincola et al., 2000). Many components of the peptide processing machinery were lost in human cancer cell lines including LMP-2, LMP-7 as well as the peptide transporter TAP1 (Restifo et al., 1993). Histological protein expression analysis of the peptide transporter chain TAP1 on different tumor types showed downregulation in 6% of the head and neck carcinoma up to 56% in melanomas (Hicklin et al., 1999; Marincola et al., 2000). Remarkably, more than 80% of the metastatic melanoma lesions and metastatic cervical carcinomas showed down regulated TAP1 expression (Hicklin et al., 1999; Marincola et al., 2000). But also patients who initially respond to immune-therapy can relapse over time due to shutdown of antigen presentation. In melanoma patients approximately one forth to one third of the patients who responded to anti-CTLA4 of anti-PD1 therapy will relapse over time (Robert et al., 2015). The cancer cells "acquire" mechanisms to resist therapy. Two recent studies showed that tumors from patients relapsing ("acquired resistance") or not responding to checkpoint inhibitor therapy ("primary resistance") had mutations in genes encoding the IFN-γ pathway, including JAK/STAT signaling (Gao et al., 2016; Zaretsky et al., 2016). The failure of these tumors to respond to IFN- γ signaling rendered them, among other effects, resistant to up-regulation of HLA-I expression and consequently less targetable for tumor-specific T cells. Several other studies recently revealed the dominance of acquired IFN-γ resistance during immunotherapy (Manguso et al., 2017; Patel et al., 2017; Sucker et al., 2017). Additionally, in another case of late acquired resistance to anti-PD1 therapy the resistant cells had a homozygous truncation mutation in β2M leading to absence of HLA-I molecules on the cell surface (Zaretsky et al., 2016). Finally, acquired resistance has also been reported in a patient with metastatic colorectal carcinoma who initially responded to TIL therapy recognizing mutated KRAS G12D presented by HLA-C*08:02 (Tran et al., 2016). The refractory tumors showed loss of HLA-C*08:02 by mutations in chromosome 6. These examples illustrate that defects in the peptide-processing pathway are an often adopted strategy of "immune resistance" where a cancer is initially recognized by the immune system but edited to evade T cell recognition

Fig. 1. Tumor immune-editing and immune defense strategies.

Cancers develop several escape mechanisms under selective pressure of T cell immunity. Tumor cells with immunogenic neo-antigens can lose their mutated epitope to evade T cell recognition. Alternatively, tumor cells can down regulate the overall expression of HLA class I molecules so immunogenic epitopes are not "visible" anymore for surveilling T cells. It has been described that 35–90% of tumors down modulate HLA class I expression. Interestingly, those HLA class I [low] tumors present a new set of tumor antigens, known as 'T cell epitopes associated with impaired peptide processing' (TEIPP). Tumor cells with complete loss of HLA class I might therapeutically be targeted by natural killer cells. This figure is adopted from reference (van der Burg et al., 2016).

(see Fig. 1). Any defect in the TAP-dependent antigen processing pathway or HLA-I gene expression will lead to failure to present conventional tumor antigens and turns these tumors 'invisible' for T-cell immunity.

4. TEIPP: tumor antigens on immune-edited cancers

Interestingly, defects in the antigen processing machinery can give rise to the presentation of a new category of tumor antigens called TEIPP: T-cell epitopes associated with impaired peptide processing. TEIPP antigens are non-mutated tumor antigens that have an alternative antigen-processing route and are only presented on tumor cells with defects in the conventional proteasome-TAP-mediated peptide-processing pathway. Presentation of this alternative peptide repertoire has been shown on cells deficient for TAP, as well as cells deficient for the ER-resident amino peptidase ERAAP (Hammer et al., 2006; van Hall et al., 2006). Targeting TEIPP antigens offers an opportunity for immune-therapy on tumor cells that adopted a resistance mechanism to evade the immune system (see Fig. 1).

The first identified mouse TEIPP tumor antigen is derived from the Trh4 protein, a ceramide synthase and fatty acid regulator that spans the ER membrane multiple times (van Hall et al., 2006). TEIPP-specific CD8 T cells were established by immunizing syngeneic mice with TAP-deficient RMA-S tumor cells expressing the co-stimulatory molecule CD80, followed by in vitro re-stimulations. Several T cell clones were isolated specific for the TAP-deficient RMA-S tumor cells and cross-reacted to non-transformed TAP-deficient B cell blasts, but not the TAP-proficient counterparts. T cells had different MHC-I restrictions. Some recognized peptide antigens when presented by the classical MHC class I molecules H-2D^b or H-2K^b as well as the conserved non-classical Qa-1^b, showing a broad T cell repertoire (van Hall et al., 2006). Using a T-cell Receptor (TCR)-transgenic mouse based on the Trh4-specific T cell clone, we showed that activated TEIPP-specific T cells could control outgrowth of MHC class I^{low} tumors (Doorduijn et al., 2017; Doorduijn et al., 2016). Importantly, no signs of adverse autoimmune reactivity was observed using these T cells, as expected since the TEIPP antigen presentation is restricted to TAP-deficient tumor cells.

5. Alternative antigen processing pathways

TAP-independent processing pathways are able to partly compensate for the loading of peptides in HLA class I molecules (Oliveira and van Hall, 2013). Although not all processing pathways of TEIPP peptides are known, several alternative peptide-processing routes have been described. Of these, the processing of signal peptides is best known. Proteins intended for cell surface display or extracellular secretion contain a signal peptide, an address code that directs their translocation into the ER lipid bilayer via the Sec61 protein-conducting channel (Voorhees and Hegde, 2016). After insertion into the protein-conducting channel, most signal peptides are cleaved from the pre-protein by the proteolytic enzyme signal peptidase (SP) (Dalbey et al., 1997). Subsequently, the signal peptide peptidase (SPP) is responsible for the intramembranous cleavage of the signal peptide stump that is trapped in the ER-membrane, which is thereby released from its intramembrane location (Weihofen et al., 2002). Peptide fragments fall into the ER lumen where they can bind HLA-I molecules (Martoglio and Dobberstein, 1998). Biochemical analysis of the peptide repertoire of TAP-deficient T2 cells confirmed that signal sequence-derived peptides are efficiently processed and presented at the cell surface of TAP-negative cells (Henderson et al., 1992; Wei and Cresswell, 1992; Weinzierl et al., 2008). The signal peptide of the human calcitonin protein encoded by the CALCA gene is an example of how peptides can be TAP-independently processed and are able to elicit an T cell immune response (El Hage et al., 2008). Interestingly, SPP-mediated liberation of peptides is not limited to signal peptides. We discovered that the prototypic TEIPP antigen in our mouse model of which the epitope is

located at the very C-terminus of the protein is also cleaved by this proteolytic enzyme (Oliveira et al., 2013).

A second mechanism that enables peptides to be loaded into MHC-I without functional TAP is operated by members of the protein convertase family, like furin (Gil-Torregrosa et al., 1998; Leonhardt et al., 2010; Medina et al., 2009). Amino acids located at the C-terminal end of secreted proteins can be cleaved by furin in the Golgi and subsequently gain access to HLA-I molecules. In our research on TEIPP antigens, we found T cell epitopes that were liberated by the proteasome, but gained access to MHC-I molecules in a TAP-independent pathway in the vesicular system (Oliveira et al., 2014). Interestingly, a surprising dominant role for metalloproteinases was found for the TAP-independent route (Lorente et al., 2012; Oliveira et al., 2014). Clearly, there are multiple roads that lead to Rome and in the absence of conventional antigen processing, an alternative peptide repertoire becomes exposed on tumor cells in the context of HLA (Oliveira and van Hall, 2015). These novel antigens and their specific T cells are therefore suitable candidates for the treatment of tumors that have escaped from conventional T-cell based immunotherapies.

6. Why are TEIPP antigens not presented by normal cells?

TEIPP antigens are derived from housekeeping proteins, expressed by all cells. But why are TEIPP antigens then only presented by TAP-deficient cells and not their TAP-proficient counterparts? Our main hypothesis is that TEIPP peptides are outcompeted in the ER by the overwhelming quantities of TAP-imported peptides. This hypothesis is supported by the fact that TAP-imported peptides are normally chaperoned into the MHC class I molecules due to the peptide loading complex (PLC). The TAP transporter is physically linked to MHC-I in the PLC via tapasin and thereby bridges these protein complexes (Neefjes et al., 2011). Thus, TAP-independent peptides that found their way into the ER via alternative mechanisms will have difficulty to find empty HLA-I molecules. In the absence of TAP, more HLA-I molecules are available for binding TEIPP peptides without the competition of the massive number of the normal peptide repertoire. We tested this notion in two systems, using the molecularly characterized mouse TEIPP derived from the Trh4 protein and human TEIPP derived from the calcitonin protein (Durgeau et al., 2011; Oliveira et al., 2011). We found that overexpression of the proteins that comprise TEIPP peptides in TAP-proficient cells resulted in MHC-I surface display of the respective TEIPP peptides, indicating that higher availability of TEIPPs in the ER successfully overcomes the peptide competition. In addition, gradual increase of TAP expression led to paralleled surface display of TEIPP, implying that the peptide transporter functions as a lever of control for the competing peptide repertoires (Durgeau et al., 2011; Oliveira et al., 2011). The alternative hypothesis that TEIPP peptides are weak binders and fail to bind HLA class I molecules in the presence of the normal repertoire was abandoned, since the Trh4 peptide is a stable and high affinity peptide that was superior to all other tested peptides (Oliveira et al., 2011). Not much is known yet on the broadness of the TEIPP repertoire, but biochemical characterization of peptides eluted from TAP-deficient cells showed a significant number of non-mutated peptides with binding affinities from high to intermediate (Weinzierl et al., 2008).

7. Determination of the TEIPP TRH4/H-2D^b structure

The Trh4 peptide in the context of the H-2D^b was crystallized in order to determine its structure (Hafstrand et al., 2016). Nearly all identified T-cell epitopes presented on H-2D^b have an asparagine at position 5 and an aliphatic amino acid at the C-terminal position, acting as main anchor positions (Falk et al., 1991; Young et al., 1994). Interestingly, the peptide sequence of the Trh4 epitope does not follow this conventional H-2D^b binding motif, but instead has several sulfur-containing amino acids: methionines at positions 1, 5, and 9 and a cysteine

at position 2. This resulted in a slightly modified positioning in the peptide-binding groove, which was found to be essential for T cell recognition. Replacement of position 5 with the traditional asparagine led to an altered binding configuration and failure of T cell activation (Hafstrand et al., 2016). Since this is the first crystal of a TEIPP antigen, its general implications remain elusive.

8. Thymic development of TEIPP T cells

T cells development takes place in the thymus and self MHC molecules shape a personalized repertoire of T cells that egress to the periphery (Breed et al., 2017; Klein et al., 2009). In the outer cortex of the thymus the immature thymocytes interact with MHC-I and -II molecules. Thymocytes that fail to interact to self MHC molecules die by neglect and those binding with a too high avidity are deleted by apoptosis to prevent the development of self-reactive T cells. Positive selection occurs by intermediate avidity interaction of thymocytes with peptide:MHC complexes. Since TEIPP antigens constitute non-mutated peptides derived from housekeeping proteins, the question arises whether the TEIPP-specific CD8 T cell repertoire is hampered by central tolerance in the thymus. Analysis of different thymic cell populations showed that indeed the Trh4 gene transcripts are expressed by medullary thymus epithelial cells (mTECs), dendritic cells as well as macrophages (Oliveira et al., 2011), as expected from a housekeeping gene. However, thymus cells of wild type, TAP-proficient mice were not recognized by the TEIPP-specific T cell clone, showing that the epitope is not presented at the surface of these cells (Oliveira et al., 2011). In agreement with this finding, we observed that a transgenic TCR efficiently mediated positive selection in the thymus of wild type mice, in contrast to the thymus of TAP-deficient mice (Doorduijn et al., 2016) (see Fig. 2). Strong and efficient thymus deletion was observed in the TEIPP-presenting latter mice.

In vitro and in vivo analysis of these TCR-transgenic T cells revealed that TEIPP T cells remained "untouched": they were naïve in the periphery and did not proliferate when transferred into wild type mice,

even under inflammatory conditions (Doorduijn et al., 2016). Importantly, the CD8 T cells exhibited full effector functions when transferred into TAP-deficient mice or when stimulated by peptide vaccination with either short or long Trh4 peptide (Doorduijn et al., 2016). These data show that in a normal, TAP-proficient environment, TEIPP-specific T cells do not become activated, unless they are specifically activated by a vaccine. Collectively, we showed that the TEIPP T cell repertoire is efficiently selected in the thymus and emerges in the periphery in a naïve state, even though the housekeeping proteins that comprise the TEIPP antigens are widely expressed. The critical step of surface display in MHC-I molecules is induced by defects in the processing pathway, like TAP-deficiency, and results in full blown activation of the T cells. Thus, TEIPP antigens are strong neoantigens, although they derive from non-mutated self-proteins.

9. Behavior of TEIPP-specific T cells in the presence of immune-edited tumors

How do TEIPP-specific T cells behave in tumor-bearing mice and how can we exploit TEIPP antigens in a therapeutic setting? One of the unique characteristics of TEIPP antigens is that they are presented on MHC-I^{low} cells, whereas the professional antigen-presenting host cells, responsible for the induction of CD8 T cell responses, have optimal processing and presentation capacities for antigens. We therefore transferred naïve TCR-transgenic TEIPP T cells in mice bearing MHC class I^{low} tumors and investigated their fate (Doorduijn et al., 2017). Interestingly, the T cells still remained naïve and tumors grew progressively. How is it possible that TAP-deficient tumors did not activate TEIPP T cells? This is probably due to the fact that TEIPP peptides are poorly cross-presented by host dendritic cells due to their alternative processing. Signal peptides of proteins are co-translationally chopped off and thus might easily be lost in the tumor debris. An elegant study demonstrated that signal peptides indeed poorly gain access to host dendritic cells and fail to induce T cell responses (Wolkers et al., 2004). Moreover, direct priming of T cells by the tumors did also not happen
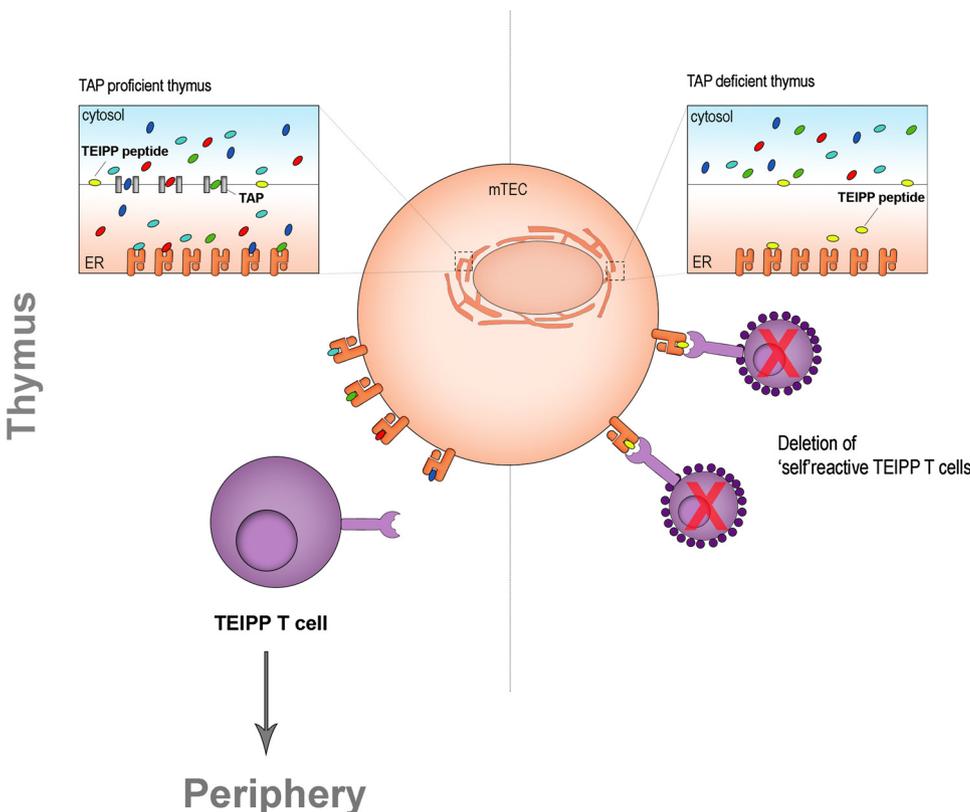

Fig. 2. Thymus selection of TEIPP T cells. TEIPP antigens are peptides from normal non-mutated self-proteins, but selectively presented in MHC class I of cell with defects in the antigen processing machinery. TEIPP epitopes are therefore not presented at the surface of medullary thymus epithelial cells and TEIPP T cells are not deleted (left side of the figure), unless the peptide transporter TAP is deleted (right side of the figure). Consequently, TEIPP-specific T cells are efficiently selected in the thymus and arrive in the periphery in a naïve state. Results of these studies were published in reference (Doorduijn et al., 2016).

because of the very low levels of MHC class I. In our recent study, we showed that enhanced expression of MHC-I molecules on the surface of tumor cells in combination with increased levels of the TEIPP tumor antigen or artificial introduction of co-stimulatory molecules resulted in efficient priming of TEIPP T cells via direct priming (Doorduijn et al., 2017). Alternatively, TEIPP T cells can be activated by peptide vaccination, which is much easier to translate to the clinic (Doorduijn et al., 2016). Once activated, TEIPP T cells potently infiltrated MHC-I^low tumors and efficiently controlled their outgrowth. In that sense, the continued naïve status of this T cell subset is an advantage, because tumor-induced suppressive mechanisms do not hold a grip on them. On the other hand, their therapeutic exploitation firstly requires priming and activation of TEIPP T cells. Due to the high specificity of TEIPP antigens on cancer cells, adoptive T cell transfer, peptide vaccination, or TCR gene transfer are all potentially effective methods to reach a safe recruitment of these immune cells.

10. Do TEIPP T cells also exist in human?

Most of our results have been obtained in mouse tumor models and, thus far, much less is known on TEIPP antigens in humans. The first molecularly identified TEIPP antigen in humans originates from the signal peptide of the CALCA gene (Durgeau et al., 2011; El Hage et al., 2008). Several CD8 T cell clones recognizing autologous tumor cells were isolated from a lung cancer patient and the peptide-epitope of one clone was derived from the non-mutated signal peptide of the pre-procalcitonin protein. Treatment of the autologous tumor cells with the proteasome inhibitor epoxomicin had no effect on the recognition and inhibition of TAP resulted in better presentation of this peptide by HLA-A2 (Durgeau et al., 2011). Conventional CD8 T cells recognizing the same lung carcinoma displayed a complete opposite reactivity pattern, showing that these two T cell clones represented complementary specificities. As far as we know, this is the first described human TEIPP antigen.

In our efforts to disclose additional human TEIPP antigens, we first utilized an approach in which dendritic cells were rendered TAP deficient by introduction of viral immune evasion proteins and incubated them with autologous T cells. Several herpes viruses encode dedicated molecules to shutdown MHC-I presentation in order to evade T cell recognition and elimination. The peptide transporter TAP is the bottleneck in the pathway and therefore an ideal target for these chronic viruses. Currently, four herpes viral genes have been described that are able to inhibit human TAP function (Ressing et al., 2013; Verweij et al., 2011). The Human Cytomegalo Virus (HCMV) US6 protein prevents ATP binding to TAP and thereby inhibits peptide translocation to the ER. ICP47 is encoded by the Herpes Simplex Virus (HSV) and prevents peptide translocation by physically obstructing the peptide-binding site of TAP. UL49.5 protein synthesized by the Bovine Herpes Virus 1 (BoHV-1) inhibits TAP by preventing structural rearrangements required for peptide transport and mediates degradation of the protein. Finally, BNLF2a encoded by Epstein Barr Virus (EBV) prevents both peptide and ATP binding to TAP.

After introduction of UL49.5 in human dendritic cells and co-culture with autologous CD8 T cells, we were able to generate TEIPP-specific T cells from all tested PBMC of healthy individuals (Lampen et al., 2010). All characterized T cell clones were reactive to target cells expressing either of the four TAP-inhibitors and were restricted by different HLA-I molecules, revealing a diverse repertoire of TEIPP antigens. The TEIPP epitopes were, however, not identified at the molecular level. In order to uncover the identity of human TEIPP we recently started a bioinformatics-based approach in which we predicted TEIPP antigens from the complete human proteome. We were able to identify 15 new TEIPP antigens presented by HLA-A2 and recognized by CD8 T cells (K.A. Marijt, unpublished data). Preliminary results revealed that some of these TEIPP-specific T cells were present in the naïve T cell repertoire, as expected based on our findings in mouse models, but others were

isolated from the antigen-experienced memory pool of T cells. This could be explained by the fact that herpes viruses like EBV and HCMV cause latent infections and are never cleared in humans. In the active episodes of infections, these viruses express their TAP-inhibiting proteins and might thereby induce TEIPP-specific T cells. However, this remains speculation at the moment and further investigation is necessary.

11. TEIPP tumor antigens and HLA-E

HLA-E and its mouse homologue Qa-1^b are non-classical MHC class I molecules and are expressed on almost all nucleated cells (Rodgers and Cook, 2005). The unique feature of HLA-E is its extremely conserved nature in the human population and across species. In contrast to the polymorphic classical class I molecules, only two functional alleles exist in humans (E*0101 and E*0103), which vary in one amino acid at a position outside the peptide-binding groove (Strong et al., 2003). HLA-E has a function in natural killer (NK)-cell biology as it is the ligand of the inhibitory receptor NKG2A/CD94. The monomorphic and conserved peptides bound in the groove of HLA-E are derived from other HLA-I molecules, thereby serving as a sensor for integrity of the antigen processing pathway. Defects in ERAAP, tapasin, proteasome and TAP all result in the failure to present the dominant monomorphic peptide (Rodgers and Cook, 2005). Therefore, inhibition of the antigen processing machinery does not only affect the expression of classical MHC-I molecules, but also strongly affects the non-classical HLA-E (van Hall et al., 2010). Instead of a monomorphic peptide, a wide array of alternative peptides emerges in HLA-E when the TAP peptide transporter is abrogated (Lampen et al., 2013). A similar alternative peptide repertoire was demonstrated in the mouse Qa-1^b in TAP-deficient tumor cells (Oliveira et al., 2010). Interestingly, these Qa-1^b presented TEIPP peptides were immunogenic and served as neoantigens (Oliveira et al., 2010; van Hall et al., 2007) and their cognate T cell specificities displayed a unique TCR composition of a shared Vα chain with a variety of Vβ chains (Doorduijn et al., 2018). Of note, inhibition of the ER-resident amino peptidase ERAAP also results in presentation of neoantigens by Qa1^b, which triggered a strong CD8 T cell response (Nagarajan et al., 2012). The conserved nature of HLA-E in the human population and the very monomorphic peptide repertoire in homeostasis renders this molecule an ideal TEIPP target for future investigations.

12. Conclusions and perspectives

Overall, our studies in both mice and man on TEIPP antigens and their cognate CD8 T cells reveal a potent and specific branch of immunity to cancers, predominantly those with defects in the antigen processing machinery (see Fig. 1). These deficiencies are rather common in human cancers and are a witness of close interactions with and selective pressure by conventional T cell immunity. TEIPP antigens are a very interesting complementary player for immunotherapy of cancer in order to include immune-edited tumors (Garrido et al., 2016; Lampen and van Hall, 2011). Cancers with a complete loss of HLA class I molecules, e.g. through loss of β2m, are not sensitive for CD8 T cells at all and need to be targeted by naturel killer cells (see Fig. 1). Compared to other categories of tumor antigens, TEIPP antigens are ideal targets: they are tumor specific, immunogenic and shared (see Table 1). TEIPP antigens presented by the conserved HLA-E molecule might represent the ultimate holy grail, especially since this class I is often upregulated by tumors (Gooden et al., 2011; Talebian Yazdi et al., 2016; van Esch et al., 2014). However, we still lack detailed knowledge of HLA-E restricted T cells and it is difficult to predict if this repertoire behaves like conventional CD8 T cells. How will we translate this concept to clinical practice? Activation and recruitment of the TEIPP T cell subset can be reached by vaccination with synthetic peptides or one could transfer cloned TEIPP TCRs. However, we would prefer to exploit the full breadth of the TEIPP antigens instead of single epitopes, and RNA-

Table 1
Overview of the categories of human tumor antigens.

Different types of tumor antigens	Examples	Description	Tumor Specific	Shared between tumors	No central tolerance	Favorable as therapeutic target
Differentiation	Tyrosinase, gp100	Expressed in tissue lineage	–	+	–	–
Overexpression	Her-2, MUC	Overexpressed in tumors	–	+	–	–
Oncoviral	E6, E7	Virus induced tumors (EBV, HPV)	+ +	+	+ +	+
Cancer testis	MAGE, NY-ESO	Expressed in testis and cancers	+	+	+	+
Neo-antigens	Point mutated epitopes	Unique for each tumor	+ +	–	+ +	+ +
TEIPP	CALCA	Induced upon defects in antigen processing	+ +	+ +	+ +	+ +

based strategies that silence TAP in host dendritic cells might be successful in reaching this goal.

Conflict of interest

The authors declared no conflict of interest.

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