Therapeutic targeting of immune escaped cancers

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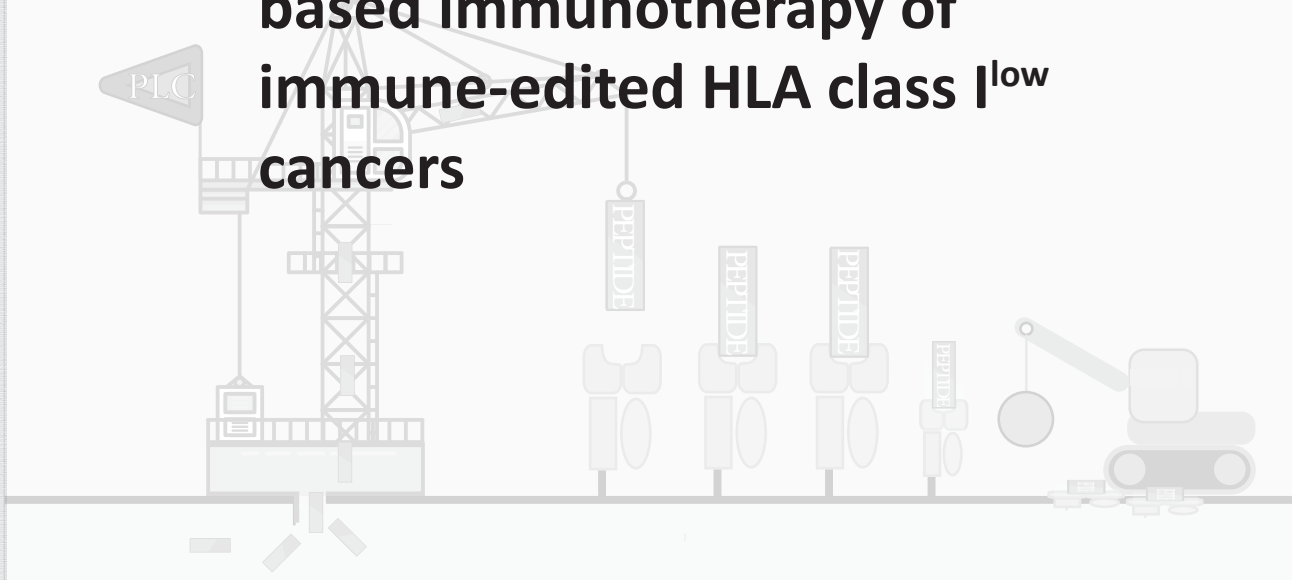
Chapter 4

TEIPP antigens for T-cell based immunotherapy of immune-edited HLA class I^{low} cancers



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Abstract

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.

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[1-4]. 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[5,6]. 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[7,8]. 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[9,10].

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[11]. 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.

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[12]. 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[13]. 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[14]. 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[15].

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[16]. 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[17,18] and checkpoint therapy-induced immune responses are frequently directed towards these personal point-mutated antigens[19-21]. Therefore this category of unique and tumor-specific T cell epitopes are at the forefront of scientific interest at the moment.

HLA class I downregulation

The vast majority of the tumor antigens described above require proteasome activity and peptide transport by TAP[22]. However, most tumors eventually develop resistance and evade immune recognition[23-25]. Immunotherapy resistance can be described by 3 categories, namely, “primary resistance”, “adaptive resistance”, and “acquired resistance”[24]. 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[7,26]. 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[27]. 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[26,27]. 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[28].

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[26,27]. Remarkably, more than 80% of the metastatic melanoma lesions and metastatic cervical carcinomas showed down regulated TAP1 expression[26,27]. 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[29]. 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[5,6]. 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[7,8,30]. 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[6]. 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[31]. 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 (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.

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[11,32]. 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).

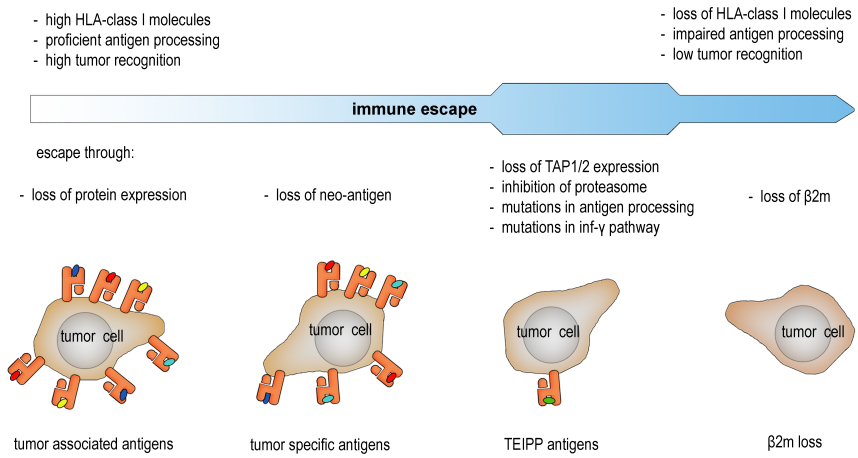

Figure 1. Tumor immune-editing and immune defense strategies.

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[74].

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[11]. 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[11]. 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 [33,34]. 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.

Alternative antigen processing pathways

TAP-independent processing pathways are able to partly compensate for the loading of peptides in HLA class I molecules[35]. 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[36]. After insertion into the protein-conducting channel, most signal peptides are cleaved from the preprotein by the proteolytic enzyme signal peptidase (SP)[37]. 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[38]. Peptide fragments fall into the ER lumen where they can bind HLA-I molecules[39]. 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[40-42]. 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[43]. 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[44].

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[45-47]. 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[48]. Interestingly, a surprising dominant role for metalloproteinases was found for the TAP-independent route[48,49]. 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[50]. 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.

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[12]. 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[51,52]. 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[51,52]. 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[51]. 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[40].

Determination of the TEIPP Trh4/H2-D^b structure

The Trh4 peptide in the context of the H-2D^b was crystallized in order to determine its structure[53]. 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[54,55]. 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[53]. Since this is the first crystal of a TEIPP antigen, its general implications remain elusive.

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[56,57]. 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[51], 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[51]. 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[33] (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[33]. 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[33]. 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.

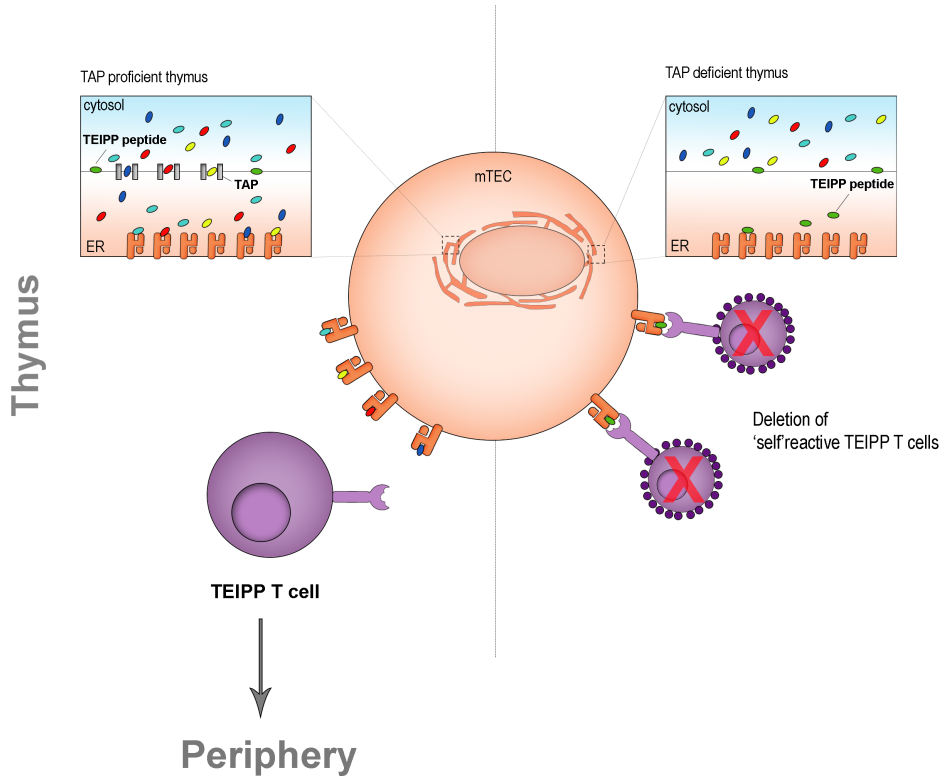

Figure 2. Thymus selection of TEIPP T cells

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[33].

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[34]. 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[58]. Moreover, direct priming of T cells by the tumors did also not happen 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[34]. Alternatively, TEIPP T cells can be activated by peptide vaccination, which is much easier to translate to the clinic[33]. 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.

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[43,52]. 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 preprocalcitonin 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[52]. 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[59,60]. 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[61]. 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.

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[62]. 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[63]. 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[62]. Therefore, inhibition of the antigen processing machinery does not only affect the expression of classical MHC-I mole-

cules, but also strongly affects the non-classical HLA-E[64]. Instead of a monomorphic peptide, a wide array of alternative peptides emerges in HLA-E when the TAP peptide transporter is abrogated[65]. A similar alternative peptide repertoire was demonstrated in the mouse Qa-1^b in TAP-deficient tumor cells[66]. Interestingly, these Qa-1^b presented TEIPP peptides were immunogenic and served as neoantigens [66,67]. T cells engaging these TEIPP peptides bound Qa-1^b molecules revealed unique TCR composition of a shared Vα chain with a variety of Vβ chains (van Hall 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[68]. 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.

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[69,70]. 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 I). 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[71-73]. 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-based strategies that silence TAP in host dendritic cells might be successful in reaching this goal.

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References

1 Larkin, J., Hodi, F. S. & Wolchok, J. D. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *The New England journal of medicine* **373**, 1270-1271, doi:10.1056/NEJMc1509660 (2015).

2 Dranoff, G. Immunotherapy at large: Balancing tumor immunity and inflammatory pathology. *Nature medicine* **19**, 1100-1101, doi:10.1038/nm.3335 (2013).

3 Delamarre, L., Mellman, I. & Yadav, M. Cancer immunotherapy. Neo approaches to cancer vaccines. *Science* **348**, 760-761, doi:10.1126/science.aab3465 (2015).

4 Chen, D. S. & Mellman, I. Elements of cancer immunity and the cancer-immune set point. *Nature* **541**, 321-330, doi:10.1038/nature21349 (2017).

5 Gao, J. *et al.* Loss of IFN-gamma Pathway Genes in Tumor Cells as a Mechanism of Resistance to Anti-CTLA-4 Therapy. *Cell* **167**, 397-404 e399, doi:10.1016/j.cell.2016.08.069 (2016).

6 Zaretsky, J. M. *et al.* Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. *N Engl J Med* **375**, 819-829, doi:10.1056/NEJMoa1604958 (2016).

7 Sucker, A. *et al.* Acquired IFNgamma resistance impairs anti-tumor immunity and gives rise to T-cell-resistant melanoma lesions. *Nat Commun* **8**, 15440, doi:10.1038/ncomms15440 (2017).

8 Manguso, R. T. *et al.* In vivo CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. *Nature* **547**, 413-418, doi:10.1038/nature23270 (2017).

9 Seliger, B. Novel insights into the molecular mechanisms of HLA class I abnormalities. *Cancer immunology, immunotherapy : CII* **61**, 249-254, doi:10.1007/s00262-011-1153-9 (2012).

10 Garrido, F., Cabrera, T. & Aptsiauri, N. "Hard" and "soft" lesions underlying the HLA class I alterations in cancer cells: implications for immunotherapy. *Int J Cancer* **127**, 249-256, doi:10.1002/ijc.25270 (2010).

11 van Hall, T. *et al.* Selective cytotoxic T-lymphocyte targeting of tumor immune escape variants. *Nature medicine* **12**, 417-424, doi:10.1038/nm1381 (2006).

12 Neefjes, J., Jongsma, M. L., Paul, P. & Bakke, O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nature reviews. Immunology* **11**, 823-836, doi:10.1038/nri3084 (2011).

13 Thomas, C. & Tampe, R. Proofreading of Peptide-MHC Complexes through Dynamic Multivalent Interactions. *Frontiers in immunology* **8**, 65, doi:10.3389/fimmu.2017.00065 (2017).

14 Melero, I. *et al.* Therapeutic vaccines for cancer: an overview of clinical trials. *Nature reviews. Clinical oncology* **11**, 509-524, doi:10.1038/nrclinonc.2014.111 (2014).

15 Morgan, R. A. *et al.* Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J Immunother* **36**, 133-151, doi:10.1097/CJI.0b013e3182829903 (2013).

16 Melief, C. J., van Hall, T., Arens, R., Ossendorp, F. & van der Burg, S. H. Therapeutic cancer vaccines. *The Journal of clinical investigation* **125**, 3401-3412, doi:10.1172/JCI80009 (2015).

17 Ott, P. A. *et al.* An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature*, doi:10.1038/nature22991 (2017).

18 Sahin, U. *et al.* Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature* **547**, 222-226, doi:10.1038/nature23003 (2017).

19 Le, D. T. *et al.* Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* **357**, 409-413, doi:10.1126/science.aan6733 (2017).

20 McGranahan, N. *et al.* Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* **351**, 1463-1469, doi:10.1126/science.aaf1490 (2016).

21 Snyder, A. *et al.* Genetic basis for clinical response to CTLA-4 blockade in melanoma. *The New England journal of medicine* **371**, 2189-2199, doi:10.1056/NEJMoa1406498 (2014).

22 Blum, J. S., Wearsch, P. A. & Cresswell, P. Pathways of antigen processing. *Annual review of immunology* **31**, 443-473, doi:10.1146/annurev-immunol-032712-095910 (2013).

23 Dunn, G. P., Bruce, A. T., Ikeda, H., Old, L. J. & Schreiber, R. D. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* **3**, 991-998, doi:10.1038/ni1102-991 (2002).

24 Sharma, P., Hu-Lieskovan, S., Wargo, J. A. & Ribas, A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell* **168**, 707-723, doi:10.1016/j.cell.2017.01.017 (2017).

25 Mittal, D., Gubin, M. M., Schreiber, R. D. & Smyth, M. J. New insights into cancer immunoediting and its three component phases--elimination, equilibrium and escape. *Current opinion in immunology* **27**, 16-25, doi:10.1016/j.coi.2014.01.004 (2014).

26 Marincola, F. M., Jaffee, E. M., Hicklin, D. J. & Ferrone, S. Escape of human solid tumors from T-cell recognition: molecular mechanisms and functional significance. *Adv Immunol* **74**, 181-273 (2000).

27 Hicklin, D. J., Marincola, F. M. & Ferrone, S. HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. *Mol Med Today* **5**, 178-186 (1999).

28 Restifo, N. P. *et al.* Identification of human cancers deficient in antigen processing. *J Exp Med* **177**, 265-272 (1993).

29 Robert, C. *et al.* Pembrolizumab versus Ipilimumab in Advanced Melanoma. *The New England journal of medicine* **372**, 2521-2532, doi:10.1056/NEJMoa1503093 (2015).

30 Patel, S. J. *et al.* Identification of essential genes for cancer immunotherapy. *Nature*, doi:10.1038/nature23477 (2017).

31 Tran, E. *et al.* T-Cell Transfer Therapy Targeting Mutant KRAS in Cancer. *The New England journal of medicine* **375**, 2255-2262, doi:10.1056/NEJMoa1609279 (2016).

32 Hammer, G. E., Gonzalez, F., Champsaur, M., Cado, D. & Shastri, N. The aminopeptidase ERAAP shapes the peptide repertoire displayed by major histocompatibility complex class I molecules. *Nat Immunol* **7**, 103-112, doi:10.1038/ni1286 (2006).

33 Doorduijn, E. M. *et al.* TAP-independent self-peptides enhance T cell recognition of immune-escaped tumors. *The Journal of clinical investigation* **126**, 784-794, doi:10.1172/JCI83671 (2016).

34 Doorduijn, E. M. *et al.* T cells specific for a TAP-independent self-peptide remain naïve in tumor-bearing mice and are fully exploitable for therapy. *OncoImmunology* (2017).

35 Oliveira, C. C. & van Hall, T. Importance of TAP-independent processing pathways. *Molecular immunology* **55**, 113-116, doi:10.1016/j.molimm.2012.10.005 (2013).

36 Voorhees, R. M. & Hegde, R. S. Structure of the Sec61 channel opened by a signal sequence. *Science* **351**, 88-91, doi:10.1126/science.aad4992 (2016).

37 Dalbey, R. E., Lively, M. O., Bron, S. & van Dijl, J. M. The chemistry and enzymology of the type I signal peptidases. *Protein Sci* **6**, 1129-1138, doi:10.1002/pro.5560060601 (1997).

38 Weihofen, A., Binns, K., Lemberg, M. K., Ashman, K. & Martoglio, B. Identification of signal peptide peptidase, a presenilin-type aspartic protease. *Science* **296**, 2215-2218, doi:10.1126/science.1070925 (2002).

39 Martoglio, B. & Dobberstein, B. Signal sequences: more than just greasy peptides. *Trends Cell Biol* **8**, 410-415 (1998).

40 Weinzierl, A. O. *et al.* Features of TAP-independent MHC class I ligands revealed by quantitative mass spectrometry. *European journal of immunology* **38**, 1503-1510, doi:10.1002/eji.200838136 (2008).

41 Wei, M. L. & Cresswell, P. HLA-A2 molecules in an antigen-processing mutant cell contain signal sequence-derived peptides. *Nature* **356**, 443-446, doi:10.1038/356443a0 (1992).

42 Henderson, R. A. *et al.* HLA-A2.1-associated peptides from a mutant cell line: a second pathway of antigen presentation. *Science* **255**, 1264-1266 (1992).

43 El Hage, F. *et al.* Preprocalcitonin signal peptide generates a cytotoxic T lymphocyte-defined tumor epitope processed by a proteasome-independent pathway. *Proc Natl Acad Sci U S A* **105**, 10119-10124, doi:10.1073/pnas.0802753105 (2008).

44 Oliveira, C. C. *et al.* New role of signal peptide peptidase to liberate C-terminal peptides for MHC class I presentation. *Journal of immunology* **191**, 4020-4028, doi:10.4049/jimmunol.1301496 (2013).

45 Gil-Torregrosa, B. C., Raul Castano, A. & Del Val, M. Major histocompatibility complex class I viral antigen processing in the secretory pathway defined by the trans-Golgi network protease furin. *J Exp Med* **188**, 1105-1116 (1998).

46 Leonhardt, R. M. *et al.* Post-endoplasmic reticulum rescue of unstable MHC class I requires proprotein convertase PC7. *Journal of immunology* **184**, 2985-2998, doi:10.4049/jimmunol.0900308 (2010).

47 Medina, F. *et al.* Furin-processed antigens targeted to the secretory route elicit functional TAP1-/-CD8+ T lymphocytes in vivo. *Journal of immunology* **183**, 4639-4647, doi:10.4049/jimmunol.0901356 (2009).

48 Oliveira, C. C. *et al.* Dominant contribution of the proteasome and metalloproteinases to TAP-independent MHC-I peptide repertoire. *Molecular immunology* **62**, 129-136, doi:10.1016/j.molimm.2014.06.006 (2014).

49 Lorente, E. *et al.* Role of metalloproteases in vaccinia virus epitope processing for transporter associated with antigen processing (TAP)-independent human leukocyte antigen (HLA)-B7 class I antigen presentation. *J Biol Chem* **287**, 9990-10000, doi:10.1074/jbc.M111.314856 (2012).

50 Oliveira, C. C. & van Hall, T. Alternative Antigen Processing for MHC Class I: Multiple Roads Lead to Rome. *Frontiers in immunology* **6**, 298, doi:10.3389/fimmu.2015.00298 (2015).

51 Oliveira, C. C. *et al.* Peptide transporter TAP mediates between competing antigen sources generating distinct surface MHC class I peptide repertoires. *European journal of immunology* **41**, 3114-3124, doi:10.1002/eji.201141836 (2011).

52 Durgeau, A. *et al.* Different expression levels of the TAP peptide transporter lead to recognition of different antigenic peptides by tumor-specific CTL. *Journal of immunology* **187**, 5532-5539, doi:10.4049/jimmunol.1102060 (2011).

53 Hafstrand, I. *et al.* The MHC Class I Cancer-Associated Neoepitope Trh4 Linked with Impaired Peptide Processing Induces a Unique Noncanonical TCR Conformer. *Journal of immunology* **196**, 2327-2334, doi:10.4049/jimmunol.1502249 (2016).

54 Falk, K., Rotzschke, O., Stevanovic, S., Jung, G. & Rammensee, H. G. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* **351**, 290-296, doi:10.1038/351290a0 (1991).

55 Young, A. C., Zhang, W., Sacchettini, J. C. & Nathenson, S. G. The three-dimensional structure of H-2Db at 2.4 A resolution: implications for antigen-determinant selection. *Cell* **76**, 39-50 (1994).

56 Klein, L., Hinterberger, M., Wirnsberger, G. & Kyewski, B. Antigen presentation in the thymus for positive selection and central tolerance induction. *Nature reviews. Immunology* **9**, 833-844, doi:10.1038/nri2669 (2009).

57 Breed, E. R., Lee, S. T. & Hogquist, K. A. Directing T cell fate: How thymic antigen presenting cells coordinate thymocyte selection. *Semin Cell Dev Biol*, doi:10.1016/j.semcdb.2017.07.045 (2017).

58 Wolkers, M. C., Brouwenstijn, N., Bakker, A. H., Toebes, M. & Schumacher, T. N. Antigen bias in T cell cross-priming. *Science* **304**, 1314-1317, doi:10.1126/science.1096268 (2004).

59 Verweij, M. C. *et al.* Inhibition of mouse TAP by immune evasion molecules encoded by non-murine herpesviruses. *Molecular immunology* **48**, 835-845, doi:10.1016/j.molimm.2010.12.008 (2011).

60 Ressing, M. E., Luteijn, R. D., Horst, D. & Wiertz, E. J. Viral interference with antigen presentation: trapping TAP. *Molecular immunology* **55**, 139-142, doi:10.1016/j.molimm.2012.10.009 (2013).

61 Lampen, M. H. *et al.* CD8+ T cell responses against TAP-inhibited cells are readily detected in the human population. *Journal of immunology* **185**, 6508-6517, doi:10.4049/jimmunol.1001774 (2010).

62 Rodgers, J. R. & Cook, R. G. MHC class Ib molecules bridge innate and acquired immunity. *Nature reviews. Immunology* **5**, 459-471, doi:10.1038/nri1635 (2005).

63 Strong, R. K. *et al.* HLA-E allelic variants. Correlating differential expression, peptide affinities, crystal structures, and thermal stabilities. *J Biol Chem* **278**, 5082-5090, doi:10.1074/jbc.M208268200 (2003).

64 van Hall, T., Oliveira, C. C., Joosten, S. A. & Ottenhoff, T. H. The other Janus face of Qa-1 and HLA-E: diverse peptide repertoires in times of stress. *Microbes Infect* **12**, 910-918, doi:10.1016/j.micinf.2010.07.011 (2010).

65 Lampen, M. H. *et al.* Alternative peptide repertoire of HLA-E reveals a binding motif that is strikingly similar to HLA-A2. *Molecular immunology* **53**, 126-131, doi:10.1016/j.molimm.2012.07.009 (2013).

66 Oliveira, C. C. *et al.* The nonpolymorphic MHC Qa-1b mediates CD8+ T cell surveillance of antigen-processing defects. *J Exp Med* **207**, 207-221, doi:10.1084/jem.20091429 (2010).

67 van Hall, T. *et al.* The varicellovirus-encoded TAP inhibitor UL49.5 regulates the presentation of CTL epitopes by Qa-1b1. *Journal of immunology* **178**, 657-662 (2007).

68 Nagarajan, N. A., Gonzalez, F. & Shastri, N. Nonclassical MHC class Ib-restricted cytotoxic T cells monitor antigen processing in the endoplasmic reticulum. *Nat Immunol* **13**, 579-586, doi:10.1038/ni.2282 (2012).

69 Lampen, M. H. & van Hall, T. Strategies to counteract MHC-I defects in tumors. *Current opinion in immunology* **23**, 293-298, doi:10.1016/j.coi.2010.12.005 (2011).

70 Garrido, F., Aptsiauri, N., Doorduijn, E. M., Garcia Lora, A. M. & van Hall, T. The urgent need to recover MHC class I in cancers for effective immunotherapy. *Current opinion in immunology* **39**, 44-51, doi:10.1016/j.coi.2015.12.007 (2016).

71 Talebian Yazdi, M. *et al.* The positive prognostic effect of stromal CD8+ tumor-infiltrating T cells is restrained by the expression of HLA-E in non-small cell lung carcinoma. *Oncotarget* **7**, 3477-3488, doi:10.18632/oncotarget.6506 (2016).

72 van Esch, E. M. *et al.* Alterations in classical and nonclassical HLA expression in recurrent and progressive HPV-induced usual vulvar intraepithelial neoplasia and implications for immunotherapy. *Int J Cancer* **135**, 830-842, doi:10.1002/ijc.28713 (2014).

73 Gooden, M. *et al.* HLA-E expression by gynecological cancers restrains tumor-infiltrating CD8(+) T lymphocytes. *Proc Natl Acad Sci U S A* **108**, 10656-10661, doi:10.1073/pnas.1100354108 (2011).

74 van der Burg, S. H., Arens, R., Ossendorp, F., van Hall, T. & Melief, C. J. Vaccines for established cancer: overcoming the challenges posed by immune evasion. *Nature reviews. Cancer* **16**, 219-233, doi:10.1038/nrc.2016.16 (2016).