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Author: Lampen, Margit H.
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Introduction

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A novel category of antigens enabling CTL immunity to tumor escape variants: Cinderella antigens

Ursula J.E. Seidel, Claudia C. Oliveira, Margit H. Lampen, and Thorbald van Hall

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1. CD8 T-cell immunity

1.1 CD8⁺ T cells

In this thesis we study CD8⁺ T cells. This subset of T cells recognizes and kills host cells, which are infected or that display changes related to transformation. They are also referred to as cytotoxic T-lymphocytes (CTLs). The T-cell receptor (TCR) of a CTL recognizes a small peptide fragment that is displayed in molecules called the class-I major histocompatibility complex (MHC-I), which are very specific and present in every nucleated cell. To recognize the peptide fragment presented in a MHC-I molecule, each TCR has to recognize the residues of the peptides presented, and to recognize residues of the MHC-molecule, which is displaying the peptide.

T cells originate in the thymus where they undergo selection before they migrate to the lymph nodes and circulate throughout the body. Selection in the thymus occurs via two subsequent processes called positive- and negative selection. During positive selection T cells are considered for their ability to recognize MHC-I molecules. If the TCR of the T cell recognizes a self-MHC-I molecule it is rescued from death by neglect. The negative selection occurs through deleting immature T cells, with receptors interacting to strong for self MHC-I complexes. This negative selection prevents contribution to auto-immunity and induces self-tolerance (1).

Once the T cells have survived both positive- and negative selection they migrate towards the lymph nodes where they resides or circulate throughout the body until they become activated.

1.2 The MHC-I molecule

The MHC-I molecule is expressed by virtually all nucleated somatic cells and consists out of two parts: β₂-microglobulin (β₂m), and the heavy chain. The heavy chain is further divided into three regions: α₁, α₂, and α₃. The α₃ region is conserved and contains the binding site for the T-cell co-receptor CD₈. The amino-terminus of α₁ and α₂ form the peptide-binding groove. This groove can bind to peptides which are generally 8-10 amino acids (aa) in length, but bulging in the middle is possible and therefore sometimes longer peptides are found (2). Peptides bind into the binding groove through so called anchor residues, which are favorable amino acids at a certain position within the presented peptide. Polymorphic residues within the α₁ and α₂ region cause differences in MHC-I molecules. Some of these residues contribute to variation in the peptide-binding groove, causing differences in the preference of the type of amino acid and position for the anchor residues. This polymorphism contributes to the large variety of MHC-I molecules found in the human population, which in its turn cause differences in the ability of humans to activate their immune system upon infection or outgrowth of tumors.

MHC-I molecules are divided in two categories: the classical MHC-I molecule (MHC-Ia), and the non-classical MHC-I molecule (MHC-Ib). Both molecules are similar in the responses that they can induce. However, they are not familial: MHC-Ia molecules are extremely polymorphic whereas MHC-Ib molecules have few alleles which are highly homologous between individuals (3, 4). The limited diversity of antigens which are presented in MHC-Ib molecules places distinct constraints on their interactions and their role in T-cell biology (4).

In humans we refer to MHC-I molecules as Human Leukocyte Antigens-I (HLA-I). The classical MHC-Ia molecules in human are referred to as HLA-A, -B, and -C, and MHC-Ib molecules are referred to as HLA-E, -F, -G, MIC and CD1. In this thesis we will only focus on the classical HLA-I molecules and the non-classical molecule HLA-E.

2. Antigen processing

MHC-I molecules present peptides at the cell-surface were they can be recognized by CTLs. Peptides presented in MHC-I, which are recognized by CTLs, are referred to as epitopes. Upon recognition of an epitope the CTL becomes activated and efficiently kills the cell. The process by which the peptides and possible epitopes are generated, and presented in MHC-I molecules, is called antigen processing. There are numerous pathways and enzymes involved, which generates and facilitates the peptides for presentation in MHC-I molecules.

2.1 Conventional antigen processing.

Peptides which are presented in the MHC-I molecules are mostly acquired in the Endoplasmatic Reticulum (ER) and are derived from endogenous origin (2). The most-defined pathway involves both the proteasome and the transporter molecule TAP. Upon entering the cytoplasm of the cell, proteins are cleaved by the proteasome, which degrades the protein into short peptide sequences. A majority of the short peptide sequences are then able to enter the ER via the Transporter associated with Antigen Processing (TAP) (figure 1a). Once in the ER, peptides which have a correct binding motive are loaded into the MHC-I molecule with help of the Peptide Loading Complex (PLC), including tapasin, calreticulin, and the thiol oxidoreductase ERp57 (2, 5, 6). The MHC-Ia molecule containing the peptide is then transported through the Golgi apparatus, towards the cell surface where it presents itself to CD8⁺ T cells (figure 1).
Figure 1: The classical HLA-I presentation pathway. Peptides derived from endogenous origin are transported through the HLA-I presentation pathway. The endogenously derived peptides are degraded by the proteasome and enter the endoplasmic reticulum through the TAP transporter after which they are loaded into HLA-Ia molecules. The HLA-I molecules are then transported through the Golgi towards the cell-surface where the can be recognized by CD8+ T-cells.

2.2 Enzymes involved in antigen processing

The proteasome is the primary source for generating peptides, which can be loaded into the MHC-I molecule. However, there are studies showing the involvement of other enzymes able to generate peptides in the cytosol. One enzyme that was described to be generating pathogen-derived peptides in the cytosol in a proteasome-independent fashion is Tripeptidyl peptidase II (TPPII) (7). This enzyme generates a HIV-Nef epitope, which is presented in both HLA-A3 and HLA-A11 molecules (7). Another enzyme is Thimet oligopeptidase (TOP) is a soluble thiol-sensitive metallopeptidase that cleaves internal bonds in peptides of 6-17 aa (8). The role of TOP is debated. TOP may play a protective role for antigenic peptides (9, 10). However, another study described that TOP actually degrades class I ligands efficiently (11), suggesting that TOP plays a role in limiting peptide availability for antigen loading (11). Regardless the function of TOP the preference for binding and trimming of peptides indicates an important role for TOP in antigen processing (8).

Recently other proteolytic enzymes like caspases 5 and -10 (12, 13), Nardilysin (14) and insulin degrading enzyme (15) are implicated to play a role in proteasome-independent antigen processing.

2.3 TAP-independent presentation pathways

Although the majority of the peptides/epitopes are processed via the conventional pathway there are several processing pathways, which are independent of TAP for peptide loading of MHC-I molecules. In this paragraph three possible TAP-independent pathways will be described: the pathway involving signal peptides, the furin mediated pathway, and the liberation of peptides from the C-terminus of ER resident proteins.

2.3.1 Signal peptides

Perhaps the most well studied TAP-independent pathway is the one that involves the enzymes Signal Peptide Peptidase (SPPase) and Signal Peptidase (SPase). Their role in the MHC-I antigen processing is just one example of how peptides are able to enter the ER without the use of TAP. Newly synthesized proteins can contain a signal sequence, which plays a key role in targeting the protein from the cytosol towards the ER-membrane (16). Once the proteins are inserted in the membrane the signal sequences can be cleaved from the precursor protein by the membrane-bound SPase. The signal peptide is then further processed by SPPase (16).

The cleavage of SPase is dependent on particular amino acids, which are present on position -1 and -3 N-terminal of the cleavage site. An amino acid with a short side chain at the -1 position, and no charged amino acids at the -3 position favor SPase cleavage. Also the length and properties of other regions in the signal sequence can influence the cleavage of SPase. Once the signal sequence is cleaved from their precursor sequence further cleavage occurs by the less well defined SPPase (16).

Peptides that after cleavage of SPase and SPPase are inside the lumen of the ER, may contain epitopes which after further processing can bind to MHC-I molecules, implicating that these epitopes are processed in a TAP-independent fashion. Two examples are a peptide derived from the interferon-inducible protein IP30 and an epitope derived from calreticulin (16, 17). Recently another epitope derived from a signal sequence was described. This epitope, derived from the signal sequence of preprocalcitonin, was a result of overexpression of the CALCA gene in lung carcinoma, indicating a possible role for epitopes derived from signal sequences in cancer immunotherapy (18).

2.3.2 Proprotein convertases: furin mediated pathway

The proprotein convertases are part of a family consisting out of six classes of proteases. There are nine proprotein convertases and they play a role in protein processing by converting an inactive precursor to its mature form, and have a role in peptide precursors trafficking through the secretory pathway. Four of the convertases are type-I membrane-bound proteases, which are: furin, PC7, the isoform PC5/6B, and SKI-1.
INTRODUCTION

Chapter 1

are mostly designated towards MHC-II molecules, in MHC-I molecules. Currently two pathways for cross-presentation are proposed which are the cytolic- and the vacuolar route. The cytolic route is a TAP-dependent process in which internalized antigens are released into the cytosol, which can then be transported to the ER by the TAP transporter and loaded into MHC-I molecules.

In the vacuolar route, antigens remain in the endocytic compartments, where they can be cleaved by proteases before being transported into MHC-I molecules. This process is independent of TAP and its underlying mechanisms are still unclear (2, 27). One question that remains is how the MHC-I molecule is transported to the endocytic compartment. A possible candidate might be the invariant chain (Ii), a chaperone molecule known for its role in the MHC-II peptide presentation pathway.

Several studies show a formation of Ii/MHC-I complexes in B-LCLs (28-30). Increasing the Ii expression through transfection also influences the MHC-I surface expression levels (31). More importantly upon Ii/MHC-I complex formation, transport towards the endocytic compartment is seen (29). The role for the Ii in the cross-presentation pathway is unconventional but recent studies show that the Ii is also responsible for transporting the TNF-related CD70 molecule towards the MHC-II compartments (32) anticipating a much broader function of the Ii than thus far described.

The association of Ii with the MHC-II is mediated via the CLIP region and it is believed that the same region is responsible for association to the MHC-I molecule (33). This matter however needs more investigation as binding towards only one MHC-I molecule has been shown (33) whereas association between Ii and MHC-I has been observed between several MHC-I molecules (28-30).

Table I: Qdm peptide sequences which bind to HLA-E (34)

| Locus | Sequence | Examples of HLA-types |
|-------|----------|-----------------------|
| HLA-A | VMAPRTLVL | A01, A03              |
|       | VMAPRTLVL | A02, A24              |
| HLA-B | VMAPRTLVL | B07, B08              |
| HLA-C | VMAPRTLVL | Cw02, Cw15            |
|       | VMAPRTLVL | Cw03, Cw04*           |
| HLA-G | VMAPRTLVL | G01                   |

a Except the Cw0402 subtype
3. HLA-E (and its mouse homolog Qa-1)

In contrast to the extreme polymorphic nature of MHC-Ia molecules, MHC-Ib molecules have few alleles and are highly homologous between humans and other species (4).

3.1 HLA-E presentation pathway

HLA-E and its mouse homolog Qa1 are, similar to MHC-Ia molecules, expressed in most tissues. Although there is only 73% of homology between both molecules, their function and non-variable peptides that they bind are surprisingly similar (34). Both molecules can bind so called signal peptides in their hydrophobic peptide-binding groove, which are mainly derived from the leader sequence of classical MHC-Ia molecules (4, 34). This set of epitopes is also referred to as Qdm (Qa-1-determinant-modifier) peptides (table I). Qdm peptides are signal sequences located at the N-terminal regions of MHC-I molecules, serving as a hydrophilic segment to co-translationally target the newly synthesized MHC-I protein to the ER. Once in the ER-membrane, the hydrophilic domain containing a Qdm peptide is released in the cytosol by enzymes called SPase and SPPase, whereas the MHC-I heavy chain is released into the ER (see figure 2) (16). This example is in contrast with the idea of §2.3.1 were the enzymes SPase and SPPase are involved in TAP-independent peptide presentation. Upon cleavage of SPase and SPPase an epitope can be either released into the ER, and therefore processed TAP-independently, or it can be released in the cytosol where it is then processed in a TAP-dependent fashion.

The domain containing Qdm, which remained in the cytosol, is processed by the proteasome into small peptide fragments and Qdm is transported into the ER via TAP. In the ER, the Qdm peptide is loaded into HLA-E and then transported to the cell-surface were it is recognized by CD94/NKG2 receptors (35, 36) (figure 2).

3.2 Natural Killer- (NK) and T-cell recognition of HLA-E

NK cells and activated CD8+ T-cells express the invariant transmembrane receptors CD94/NKG2 (34, 37). The CD94 subunit makes the most contact with the HLA-E molecule and the presented peptide, whereas the NKG2 subunit is responsible for the transduction of signals (38-40). NKG2 receptor genes are clustered in the NK-locus and there are different variants: NKG2A, -B, -C, -D, -E and -H. Of these six, only the NKG2D does not form a heterodimer with the CD94 complex and NKG2D is also clearly distinct from NKG2A, -B, -C, -E and -H; which differ by only a few amino acids (37). NKG2A and NKG2B are alternative splice products of one gene in which NKG2B appears to be in disadvantage. Also NKG2E and NKG2H are alternative splice products of one gene (41). NKG2A, -C and -E play a role during recognition of HLA-E and small differences in the NKG2A, -C, and -E receptors determines its affinity to the CD94 receptor. These differences also determine whether the CD94/NKG2D heterodimer acts as an activating or inhibitory receptor. NKG2C and NKG2E are both activating receptors and are in general less frequently expressed than NKG2A, which provides inhibitory signals (41, 42).

NK cells are part of the so-called innate immunity, which are the first line of defense against infections or transformation. If due to these alterations the cells fail to present MHC-Ia molecules at the cell-surface the NK cells will be activated and lyse the cells. NK cells which express either CD94/NKG2A or CD94/NKG2C can engage with HLA-E and the MHC-Ia Qdm peptide and provide either an activating (NKG2C) or inhibitory (NKG2A) signal, which contributes together with other signals to determine whether the NK cell should become active.
Apart from NK-mediated recognition, TCR-mediated recognition of HLA-E or its mouse homolog Qa-1β has previously been described (43-47). Upon cellular stress the Qdm peptide is replaced by novel much diverse repertoire of peptides (34, 43-47). Epitopes derived from Epstein bar virus (EBV) (43), Mycobacterium Tuberculosis (44), and mycobacteria (47) have been reported to be presented in HLA-E where then can be recognized by CD8+ T cells. Romagnani et al reported that allogeneic T-cells might also recognize peptides in HLA-E, which are not present in the host of origin (46). This makes HLA-E a target for possible implications upon transplantation, or can be involved in anti tumor immune responses. Interestingly, Oliveira et al reported Qa-1β restricted CTLs that recognized peptides presented solely on a variety of TAP-impaired tumor cells, so called TEIPPs (45). As Qa-1β is highly homologous to HLA-E, this finding could imply that this type of CTLs can also exist in the human population thereby opening new possibilities to treat tumors with antigen processing defects (4, 45).

3.3 HLA-E expression by tumors

Almost all nucleated cells express HLA-E and a majority of tissues from healthy donors show weak to moderate staining for HLA-E. Staining of HLA-E is strong in subsets of lymphoid cells, epididymis and the adrenal gland. HLA-E expression is not detected in the liver, pancreas, myocytes and the central nerve system (CNS) (http://www.proteinatlas.org) (48).

It has been observed that in sections of melanomas variable fractions of HLA-E expression were found ranging form 30-70% on primary tumors and 10-20% on metastatic lesions (49). In colorectal cancers, and astrocytic tumors, strong HLA-E expression was observed, while its healthy counterpart showed weak staining of HLA-E (50, 51). In cervical carcinoma, a difference in HLA-E expression was observed between invasive- and non-invasive cancers; the more invasive the cancer the greater the HLA-E expression (52). A recent study reports HLA-E expression in early breast carcinoma patients (53). They observed that 50% of the breast carcinoma samples have expression of HLA-E. Remarkably, patients whose tumors had a complete loss of the classical HLA-I molecules, but coinciding HLA-E expression had a worse relapse free period. Also an association is seen between classical HLA-I processing pathway and HLA-E expression (53). The increased expression of HLA-E on tumors is not fully understood.

In vitro cultured melanoma- and colorectal cancer cell-lines display low but significant amounts of HLA-E on the cell surface. However in these cell-lines HLA-E expression is clearly detected intra-cellular. Upon IFNγ treatment an increase of HLA-E is observed at the cell-surface (49, 50, 54) resulting in a decreased susceptibility to CTL lysis of CTL expressing the inhibitory receptor CD94/NKG2a (49, 55). In one study an imbalance of MHC-Ia heavy chain/β2m expression can modulate HLA-E surface expression, meaning that expression of HLA-E correlates inversely with the expression of MHC-Ia molecules in vitro cultures (54). However, for several studies on other tumor cell lines don’t observe such an inverse correlation (49, 56). It must also be noted that the general low HLA-E expression in tumor cell-lines observed in vitro contrasts with the in general increased HLA-E expression in site using immunohistochemistry.

4. Inhibition of the conventional MHC-I pathway upon viral infection.

Upon viral infection of a cell, the infected cells start to produce viral proteins. Like other proteins, the viral proteins are also processed via the antigen processing pathway by viral proteins. The family of Herpesviridae has adapted numerous ways to avoid recognition by CTL. Depicted are the viral proteins, which are inhibiting molecules involved in MHC-I antigen processing and the virus they are derived from. Figure adapted from (57)
the TAP transporter molecule: UL49.5, ICP47, US6, BNLF2a and V012 (figure 3). For four of these TAP inhibitors their mechanism of action is unraveled. UL49.5 is a protein belonging to the genus Varicellovirus and for the bovine herpesvirus-1 (BHV-1) variant it is known to efficiently inactivate TAP by arresting it in a translocation-incompetent state; and promoting the degradation of TAP (58-60). ICP47 is found in HSV type 1 and 2 where binds to the cytosolic site of the TAP complex, thereby acting as a high-affinity competitor for peptide binding (61-64). The human HCMV protein US6 is an ER-resident protein and it prevents ATP binding to TAP, thereby blocking the peptide transport (65, 66). The viral TAP-inhibitor BNLF2a is encoded by EBV and prevents both peptide and ATP binding to TAP (67, 68). The latest identified TAP-inhibitor V012, is found in cowpox virus (CPX) and is the first TAP inhibitor outside the family of Herpesviridae. Its TAP-inhibiting mechanisms has not yet been identified (57, 69, 70).

5. Immune escape of tumors

Like viruses, tumors have adopted mechanisms to prevent peptide presentation by MHC-Ia. These mechanisms can be divided into two categories to which Garrido et al refers to as “hard” or “soft” defects (71). Both “hard” and “soft” lesions are associated with poor prognosis as it leads to failure of recognition and elimination by tumor specific CTLs, as the expression of MHC-I molecules at the cell-surface is decreased (72-74). “Hard” defects are irreversible and structural such as loss of gene copies via loss of heterozygosity (LOH), mutations and deletions of MHC-Ia heavy chain genes or the β2m gene. These mutations and

Figure 4: The “hard”- and “soft” defects in tumor tissue. Tumors can escape immune recognition through numerous ways. “Hard” defects are irreversible such as mutations and deletions of MHC-Ia heavy chain genes or the β2m gene or LOH. Soft” defects are reversible regulatory defects such as downregulation of MHC-Ia transcription due to, hypermethylation of the promoters, oncogene activation, and or blocking of the Jack-STAT pathway.

presentation pathways and can be presented at the cell-surface. If a cell presents such viral proteins at the cell-surface they can be recognized by CTLs, becomes activated, and kills the infected cells. To avoid this recognition, most DNA viruses express so-called immune evasion proteins. The protein prohibits presentation of viral proteins in MHC-I molecules at the cell-surface, thereby avoiding recognition by the CTLs and allowing viruses to persist in the human body.

The most well studied viruses and their immune evasion proteins are from the family of Herpesviridae and most people are infected with one or more of the eight known Herpesviruses: herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), human cytomegalovirus (HCMV), human herpesviruses 6 and 7 (HHV-6 and HHV-7), Epstein-Barr virus (EBV), and Kaposi’s sarcoma-associated herpesvirus (KSHV) (57). Due to their numerous strategies to prevent peptide presentation of the MHC-I molecule capability the family of Herpesviridae causes life-long persistent infections in humans. For example the viral derived proteins US2, US10, US11 (HCMV), mouse cytomegalo virus (MCMC) glycoprotein (gp) 48, and murine γ-herpesvirus 68 (MHV68) mK3, target MHC-I molecules in a selective fashion thereby accelerated degradation of MHC-I molecules (57). Other mechanisms include intracellular retention (US3 (HCMV) and gp40 (MCMV)) or increased endocytosis (kK3/kK5 (KSHV) and BILF1 (EBV)) of MHC-I molecules, as well as interference with the recognition of peptide loaded MHC-I by CTLs (gp34 (MCMV)) (57). All MHC-I interfering proteins and their origin are summarized in figure 3.

Among the proteins involved in immune escape there are five viral-inhibitors known to impair the function of the TAP transporter molecule: UL49.5, ICP47, US6, BNLF2a and V012 (figure 3). For four of these TAP inhibitors their mechanism of action is unraveled. UL49.5 is a protein belonging to the genus Varicellovirus and for the bovine herpesvirus-1 (BHV-1) variant it is known to efficiently inactivate TAP by arresting it in a translocation-incompetent state; and promoting the degradation of TAP (58-60). ICP47 is found in HSV type 1 and 2 where binds to the cytosolic site of the TAP complex, thereby acting as a high-affinity competitor for peptide binding (61-64). The human HCMV protein US6 is an ER-resident protein and it prevents ATP binding to TAP, thereby blocking the peptide transport (65, 66). The viral TAP-inhibitor BNLF2a is encoded by EBV and prevents both peptide and ATP binding to TAP (67, 68). The latest identified TAP-inhibitor V012, is found in cowpox virus (CPX) and is the first TAP inhibitor outside the family of Herpesviridae. Its TAP-inhibiting mechanisms has not yet been identified (57, 69, 70).

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Two recent case studies show a clear association between low HLA-I expression and progression of tumors after immunotherapy (85, 86). In both studies metastatic lesions were isolated from a melanoma patient treated with autologous melanoma cell vaccine (M-VAX) and interferon-α2b. After treatment eight of the metastatic lesions regressed in the treated patient, while two lesions progressed. Studying the HLA-I surface levels it was seen that the progressive lesions had low HLA-I surface expression while the regressing lesions maintained their HLA-I surface levels (85, 86). A decrease in HLA-I surface expression prevents expression of tumor-specific antigens on the cell-surface thereby avoiding CTL recognition.

### 6. Tumor antigens

Tumor antigens, which are expressed at the surface of a tumor cell, are divided in different categories based on the proteins they originate from. These categories are:

- **Viral epitopes**: expressed by all virus-induced tumors e.g. Burkitt lymphoma, nasopharyngeal carcinoma, and cervical cancers, and 15-20% of tumors is virally induced. Cervical cancer is virtually all cases caused by the Human Papilloma (81).

- **Point mutations**: found in microsatellite unstable tumors. This is associated with increased expression of HLA-I (82).

- **Differentiation antigens**: expressed by tumors during differentiation. They are found in squamous cell carcinoma, breast cancer, and melanoma.

- **Cancer testis antigens**: expressed by tumor-specific cells and not by normal cells. They are found in testicular cancer and melanoma.

- **Cryptic epitopes**: found in tumors that have undergone chromosomal deletions. They are found in glioblastoma and renal cell carcinoma.

The occurrence of either LOH or alterations in the expression of the PLC and TAP has been thoroughly investigated. LOH is studied in a wide variety of tumor types and is mostly located on either the chromosomes harbouring the HLA-A/B/C genes or the β2m gene (71). Studies that focus on LOH in tumors are summarized in table II. In short: LOHs are present at different chromosomes and differ between 11-90% depending on which microsatellite used. Moreover, no significant difference is seen between primary and metastatic lesions (77-83).

The loss or downregulation of TAP is studied in a wide variety of tumors and its metastasis (72, 84). The alteration of TAP levels is found in all sorts of tumors but its frequency varies from 10% for Head and neck squamous cell carcinoma (HNSCC) up to 74% in renal cell carcinoma (72). Moreover the frequency of TAP loss is increased in metastatic lesions (84).

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Virus and known epitopes are derived from the E6 and E7 oncoproteins (87, 88). Burkitt lymphoma, Nasopharyngeal carcinoma, and Hodgkin lymphoma are caused by EBV, and expression of the EBV specific proteins; EBNA-1, LMP-1 and LMP2 have been observed in tumor cells (89).

The majority of cancer antigens are caused by point mutations in constitutive cellular proteins. CTL responses against e.g. Ras protein have been reported in breast carcinoma (90). Moreover, in vitro CTL responses derived from healthy donors against p53 have been reported (91) and p53 is frequently mutated and/or overexpressed in numerous cancers. Several phase I/II immunization trials have been conducted using p53 as a target (92). However, thus far therapeutic vaccination strategies targeting p53 have not shown consistent or convincing clinical efficiency (92), and therefore improvement in the efficiency of the immunogenic response against p53 is desirable.

Other mutations such as in MUM-1 and CDK4 have shown to lead to presentation of epitopes in MHC-I molecules (90). Some point mutations may reflect changes contributing to neoplastic transformation, while other mutations may reflect the genetic instability of tumors. These mutations and their effects are patient specific.

Differentiation antigens are selectively expressed in certain normal tissues and, therefore, also in tumors derived from these tissues. Most described epitopes were detected as targets for CTL in patients with melanoma and are usually not mutated (89, 90, 93). Three differentiation antigens are expressed in more than 90% of human melanoma namely MART-1, tyrosinase, and gp100 and it is known that melanoma patients have CTLs capable of recognizing these antigens (93).

Several tumors, e.g. melanoma, bladder carcinoma, lung carcinoma and liver carcinoma, express genes which are normally only expressed during embryonic development (94). The epitopes representing this group are referred to as cancer/testis antigens, defined on the basis of their normal tissue specific expression in testis or ovary germ-line cells (89, 90, 93, 94). CTLs directed against these antigens have been identified and as they are expressed in a wide variety of tumors, they form an attractive group to use in tumor vaccination strategies.

The last group of tumor antigens, cryptic epitopes, is derived from aberrant gene transcripts or unusual translation products (95-97). Most of these translation products appear to be tumor-specific, are not detected in normal cells, and are caused through alternative open reading frames, translated introns or pseudo-genes.

Even though there has been a large number of tumor antigens identified, T cell based immunotherapy suffers from a discrepancy between the induction of tumor-specific immune responses in experimental settings and therapeutic immunity in clinical relevant conditions (98).

A problem might arise when tumors impair their antigen presentation pathway. In this case, the presentation at the cell surface of the classical tumor antigens is decreased or even lost (99). Studies have shown that the residual peptides presented on TAP-impaired cells are dramatically different than the normal peptide repertoire (17, 100, 101). However, this alteration leads to presentation of new self-immunogenic peptides, which can be recognized by CTLs, thereby eradicating the tumor (45, 102, 103). These newly presented immunogenic peptides represent a novel category of tumor antigens, called TEIPP.

### Table III: Categories of human tumor antigens recognized by CTL

| Category          | Description                                           | Examples               |
|-------------------|-------------------------------------------------------|------------------------|
| Viral antigens    | Virus-induced tumors (e.g. EBV, HPV)                  | EBNA-1, E6, E7         |
| Point mutations   | Unique for each tumor                                 | MUM-1, CDK-4, p53, Caspase-8 |
| Differentiation   | Expessed in tissue lineage                            | Tyrosinase, GP100, Mart-1 |
| Cancer testis     | Largely expressed during development and cancers      | MAGE, NYO-ESO-1        |
| Cryptic epitopes  | Associated with aberrant transcription and translation| RU2, GnT-V, HPX42B      |
| TEIPP             | Associated with antigen processing defects            | CALCA                  |
7. T-cell Epitopes associated with Impaired Peptide Processing (TEIPP)

TEIPPs are like most tumor antigens not presented at the cell-surface of healthy tissue. However, unlike the other classes of tumor antigens, TEIPPs are presented on tumors that have an impaired antigen presentation pathway. Moreover, TEIPPs are derived from housekeeping proteins and are expressed by a wide variety of cells (103, 104). CTLs recognizing TEIPP display preferential killing towards antigen-impaired tumors and it has been demonstrated that TEIPP T-cell receptors engage with epitopes that are displayed in the residual MHC-I molecules (103).

So far, studies on identifying TEIPPs and their CTLs have been limited to mouse models (45, 102-104) and only one TEIPP has been fully identified namely the Trh4-epitope (also known as Lass5), which is presented in H-2D\(^b\) molecules, a classical MHC-Ia in the mouse. There are also TEIPP-CTLs found directed against TAP-independent epitopes presented in the non-classical Qa-1, which is the mouse homolog of HLA-E (45). However, the exact epitopes have not been identified.

The Trh4 epitope is derived from the C-terminus of the Trh4-protein, which is an ER membrane spanning protein. TAP\(^+\) RMA cells were not recognized by the Trh4-CTL whereas the TAP-negative counterpart RMA-S was efficiently lysed, demonstrating that the Trh4-epitope is processed in a TAP-independent fashion. As expression of the Thr4 protein was similar in both cells, it was hypothesized that the Trh4 epitope is not presented in TAP\(^+\) RMA due to the large quantities of peptide transported into the ER by TAP, thereby creating an unfavorable environment for the TAP-independent Trh4-epitope to be loaded in H-2D\(^b\) molecules (103). Further study shows that overexpression of Trh4 epitope in TAP\(^+\) cells resulted in recognition and lysis by the Trh4-CTL illustrating that indeed the influx of TAP-transported peptides create an efficient barrier to inhibit loading of peptides from alternative processing routes. The impairment of TAP function lowers this resistance allowing the MHC-I presentation of other peptide sources (104).

The Trh4-protein belongs to a family of fatty-acid regulators and these housekeeping proteins are expressed by a wide variety of cells (103). Indeed, tissues derived from TAP1 knockout mice were recognized by the Trh4-CTL apart from the spleen and liver. As expected, none of the tissues derived from wild-type mice were recognized (104). This shows that the Trh4-epitope can be applied to treat a wide variety of processing deficient tumors and limits possible side-reactivity as healthy TAP\(^+\) cells are not recognized (103). Although the reactivity of TEIPP CTLs resembles that of natural killer cells, TEIPP antigens behave like conventional tumor antigens for CTL.

The induction of TEIPP CTLs has been investigated in a mouse model in numerous ways: vaccination with the Trh4 short peptide, cellular vaccination with TAP-deficient dendritic cells and adoptive transfer of \textit{in vitro} expanded CTLs (102, 103). In all three cases the TEIPP-targeted immunotherapy resulted in clearance of tumors with MHC-I defects, in particular TAP deficiencies. Inducing TEIPP-specific CTLs might provide the first local immune activation and cytokine release upon recognition of antigen-impaired lesions.

8. Scope of thesis

This thesis focuses on alternative antigen presentation in the context of tumors with defects in antigen processing. In chapter 2, we investigate whether the human T-cell repertoire harbors a population reactive to TAP-impaired targets. The detection of such T-cell reactivity would indicate that, similar to mice, the human system contains a TEIPP-specific T-cell repertoire. To identify TEIPP antigens in humans, we set up a bioinformatics approach to predict HLA-I binding peptides with potential TEIPP characteristics in chapter 3. We made a start with a screening method to determine the immunogenicity of these self-peptides. An attractive strategy towards the development of immune-therapy based on TEIPPs is to identify TEIPPs in the human HLA-E molecule. This non-classical HLA-I molecule has only two variants among the human population. As these molecules only differ by one amino acid, the presented peptides are the same among humans. Moreover, HLA-E is frequently preserved or over-expressed in tumors. To gain a more profound insight in the peptide repertoire presented by the non-classical molecule HLA-E under conditions were TAP is impaired; we performed peptide elutions in chapter 4. Since these peptides derived from the elution are based natural selection through antigen presentation we also determine a peptide-binding motif for HLA-E. The implications of increased HLA-E expression in ovarian- and cervical carcinoma patients are assessed in chapter 5, in which we also study the presence of T-cells containing CD94/NKG2 receptors which are able to interact with HLA-E. It has been described in literature that the invariant chain interacts with HLA-I molecules, especially in the absence of TAP. This prompted us to test the idea that this protein-protein complex is implicated in the HLA-I...
presentation of TEIPPs. In chapter 6 we studied the surface expression of the CLIP peptide, which is derived from the invariant chain, in leukemia patients. We confirmed the interaction of the invariant chain with HLA-I molecules in leukemic cells, and surprisingly found that the CLIP peptide promiscuously binds HLA-I molecules, maybe as a result of this interaction. The existence and nature of human TEIPP antigens is reviewed in the discussion where we also give an overview on the current therapies aiming at counteracting MHC-I deficiencies in tumors.

Reference List
1. von Boehmer H. 2004. Selection of the T-cell repertoire: receptor-controlled checkpoints in T-cell development. Advances in immunology 84: 201-38
2. Jensen PE. 2007. Recent advances in antigen processing and presentation. Nat Immunol 8: 1041-8
3. Hofstetter AR, Sullivan LC, Lukacher AE, Brooks AG. 2011. Diverse roles of non-diverse molecules: MHC class Ib molecules in host defense and control of autoimmunity. Current opinion in immunology 23: 104-10
4. Rodgers JR, Cook RG. 2005. MHC class Ib molecules bridge innate and acquired immunity. Nat Rev Immunol 5: 459-71
5. Endert P. 2008. Role of tripeptidyl peptidase II in MHC class I antigen processing - the end of controversies? Eur J Immunol 38: 609-13
6. Vyas JM, Van der Veen AG, Ploegh HL. 2008. The known unknowns of antigen processing and presentation. Nat Rev Immunol 8: 607-18
7. Seifert U, Maranon C, Shmueli A, Desoutter JF, Wesoloski L, Janek K, Henklein P, Diescher S, Andrieu M, de la Salle H, Weinschenk T, Schild H, Laderach D, Galy A, Haas G, Kloetzel PM, Reiss Y, Hosmalin A. 2003. An essential role for tripeptidyl peptidase in the generation of an MHC class I epitope. Nature immunology 4: 375-9
8. Saveanu L, Fruci D, van Endert P. 2002. Beyond the proteasome: trimming, degradation and generation of MHC class I ligands by auxiliary proteases. Molecular immunology 39: 203-15
9. Portaro FC, Gomes MD, Cabrera A, Fernandes BL, Silva CL, Ferro ES, Juliano L, de Camargo AC. 1999. Thimet oligopeptidase and the stability of MHC class I epitopes in macrophage cytosol. Biochemical and biophysical research communications 255: 596-601
10. Silva CL, Portaro FC, Bonato VL, de Camargo AC, Ferro ES. 1999. Thimet oligopeptidase (EC 3.4.24.15), a novel protein on the route of MHC class I antigen presentation. Biochemical and biophysical research communications 255: 591-5
11. Saric T, Beninga J, Graef CI, Akopian TN, Rock KL, Goldberg AL. 2001. Major histocompatibility complex class I-presented antigenic peptides are degraded in cytosolic extracts primarily by thimet oligopeptidase. The Journal of biological chemistry 276: 36474-81
12. Lopez D, Garcia-Calvo M, Smith GL, Del Val M. 2010. Caspases in virus-infected cells contribute to recognition by CD8+ T lymphocytes. Journal of immunology 184: 5193-9
13. Lopez D, Jimenez M, Garcia-Calvo M, Del Val M. 2011. Concerted antigen processing of a short viral antigen by human caspase-5 and -10. The Journal of biological chemistry 286: 16910-3
14. Kessler JH, Khan S, Seifert U, Le Gall S, Chow KM, Paschen A, Bres-Vloemans SA, de Ru A, van Montfoort N, Franken KL, Benkhuysen WE, Brooks JM, van Hall T, Ray K, Mulder A, Doxiadis, II, van Swieten PF, Overkleeft HS, Prat A, Tomkinson B, Neefjes J, Kloetzel PM, Rodgers DW, Hersh LB, Drijfhout JW, van Veenen PA, Ossendorp F, Melief CJ. 2011. Antigen processing by nardiysin and thimet oligopeptidase generates cytotoxic T cell epitopes. Nature immunology 12: 45-53
15. Parmentier N, Stroobant V, Colau D, de Diesbach P, Morel S, Chapipo J, van Endert P, Van den Eynde B.J. 2010. Production of an antigenic peptide by insulin-degrading enzyme. Nature
Sugita M, Brenner MB. 1995. Association of the invariant chain with MHC class I molecules: preference for HLA class Ibeta 2-microglobulin heterodimers, specificity, and influence of the MHC peptide-binding groove. *Journal of immunology* 157: 4503-10

Reber AJ, Turnquist HR, Thomas HJ, Lutz CT, Solheim JC. 2002. Expression of invariant chain can cause an allele-dependent increase in the surface expression of MHC class I molecules. *Immunogenetics* 54: 74-81

Zwart W, Peperzak V, de Vries E, Keller AM, van der Horst G, Veraa EA, Geumann U, Janssen H, Janssen L, Naik SH, Neefjes J, Borst J. 2010. The invariant chain transports TNF family member CD70 to MHC class II compartments in dendritic cells. *Journal of cell science* 123: 3817-27

Powis SJ. 2006. CLIP-region mediated interaction of Invariant chain with MHC class I molecules. *FEBS letters* 580: 3112-6

van Hall T, Oliveira CC, Joosten SA, Ottenhoff TH. 2010. The other Janus face of QA-1 and HLA-E: diverse peptide repertoires in times of stress. *Microbes Infect* 12: 910-8

Braud VM, Allan DS, O'Callaghan CA, Soderstrom K, D'Andrea A, Ogg GS, Lazetic S, Young NT, Bell Jl, Phillips JH, Lanier LL, McMichael AJ. 1998. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 391: 795-9

Vance RE, Jamieson AM, Raulet DH. 1999. Recognition of the class Ib molecule Qa-1(b) by putative activating receptors CD94/NKG2C and CD94/NKG2E on mouse natural killer cells. *The Journal of experimental medicine* 190: 1801-12

Houchins JP, Yabe T, McSherry C, Bach FH. 1991. DNA sequence analysis of NKG2, a family of related CDNA clones encoding type II integral membrane proteins on human natural killer cells. *The Journal of experimental medicine* 173: 1017-20

Kaiser BK, Pizarro JC, Kerns J, Strong RK. 2008. Structural basis for NKG2A/CD94 recognition of HLA-E. *Proceedings of the National Academy of Sciences of the United States of America* 105: 6696-701

Petrie EJ, Clements CS, Lin J, Sullivan LC, Johnson D, Huyton T, Heroux A, Hoare HL, Beddoe T, Reid HH, Wilce MC, Brooks AG, Rossjohn J. 2008. CD94/NKG2A recognition of human leukocyte antigen (HLA)-E bound to an HLA class I leader sequence. *The Journal of experimental medicine* 205: 725-35

Sullivan LC, Clements CS, Beddoe T, Johnson D, Hoare HL, Lin J, Huyton T, Hopkins EJ, Reid HH, Wilce MC, Kabat J, Borrego F, Coligan JE, Rossjohn J, Brooks AG. 2007. The heterodimeric assembly of the CD94-NKG2 receptor family and implications for human leukocyte antigen-E recognition. *Immunity* 27: 900-11

Borrego F, Masilamani M, Marusina AI, Tang X, Coligan JE. 2006. The CD94/NKG2 family of receptors: from molecules and cells to clinical relevance. *Immunologic research* 35: 263-78

Vales-Gomez M, Reynburn HT, Erskine RA, Lopez-Botet M, Strominger JL. 1999. Kinetics and peptide dependency of the binding of the inhibitory NK receptor CD94/NKG2-A and the activating receptor CD94/NKG2-C to HLA-E. *The EMBO journal* 18: 4250-60

Garcia P, Llano M, de Heredia AB, Willberg CB, Caparros E, Aparicio P, Aparicio P, Braud VM, Lopez-Botet M. 2002. Human T cell receptor-beta 2-microglobulin binding of the inhibitory NK receptor CD94/NKG2-A and the activating receptor CD94/NKG2-C to HLA-E. *The EMBO journal* 18: 4250-60

Heinzel AS, Grotzkje JE, Lines RA, Lewinsohn DA, McNabb AL, Streblow DN, Braud VM, Grieser HJ, Belisle JT, Lewinsohn DM.

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2002. HLA-E-dependent presentation of Mtb-derived antigen to human CD8+ T cells. J Exp Med 196: 1473-81
45. Oliveaira CC, van Velen PA, Querido B, de Ru A, Sluijter M, Laban S, Drijhout JW, van der Burg SH, Offringa R, van Hall T. 2010. The nonpolymorphic MHC Qa-1b mediates CD8+ T cell surveillance of antigen-processing defects. J Exp Med 207: 207-21
46. Romagnani C, Pietra G, Falco M, Millo E, Mazzarino P, Biassoni R, Moretta A, Moretta L, Mingari MC. 2002. Identification of HLA-E-specific alloreactive T lymphocytes: a cell subset that undergoes preferential expansion in mixed lymphocyte culture and displays a broad cytolytic activity against allogeneic cells. Proc Natl Acad Sci U S A 99: 11328-33
47. Joosten SA, van Meijgaardan KE, van Weeren PC, Kazi F, Geluk A, Savage ND, Drijhout JW, Flower DR, Hanekom WA, Klein MR, Ottenhoff TH. 2010. Mycobacterium tuberculosis peptides presented by HLA-E molecules are targets for human CD8+ T-cells with cytotoxic as well as regulatory activity. PLoS Pathog 6: e1000782
48. Uhlen M, Oksvold P, Fagerberg L, Lundberg D, Jonasson K, Borg M, Zwahlen M, Kampf C, Wester K, Hober S, Werners H, Bjorling L, Ponten F. 2010. Towards a knowledge-based Human Protein Atlas. Nature biotechnology 28: 1248-50
49. Derre L, Corvaisier M, Charreau B, Moreau A, Godefroy E, Moreau-Aubry A, Joteureau F, Gervois N. 2006. Expression and release of HLA-E by melanoma cells and melanocytes: potential impact on the response of cytotoxic effector cells. J Immunol 177: 3100-7
50. Levy EM, Bianchini M, Von Euv EM, Barrio MM, Bravo AI, Furnan D, Domenichini E, Magano C, Pinsky V, Zucchini C, Valvasori L, Mordoh J. 2008. Human leukocyte antigen-E protein is overexpressed in primary human colorectal cancer. Int J Oncol 32: 633-41
51. Mittelbronn M, Simon P, Loffler C, Bunz B, Harter P, Schlaszus H, Schleich A, Tabata C, Goepfert B, Meyermann R, Weller M, Wischhusen J. 2007. Elevated HLA-E levels in human glioblastomas but not in grade I to III astrocytomas correlate with infiltrating CD8+ T-cells. J Neuroimmunol 189: 50-8
52. Goncalves MA, Le Discorde M, Simoes RT, Rabreau M, Soares EG, Donadi EA, Carosella ED. 2008. Classical and non-classical HLA molecules and p16(INK4a) expression in precursors lesions and invasive cervical cancer. Eur J Obstet Gynecol Reprod Biol 141: 70-4
53. de Krijff EM, Sajet A, van Nels JG, Natanov R, Putter H, Smit VT, Liebers GJ, van den Elsen PJ, van de Velde CJ, Kuppen PJ. 2010. HLA-E and HLA-G expression in classical HLA class I-negative tumors is of prognostic value for clinical outcome of early breast cancer patients. J Immunol 185: 7452-9
54. Marin R, Ruiz-Cabello F, Pedrinaci S, Mendez R, Jimenez P, Geraghty D, Garrido F. 2003. Analysis of HLA-E expression in human tumors. Immunogenetics 54: 767-75
55. Malmberg KJ, Levitsky V, Norell H, de Matos CT, Carlsten M, Schedvins K, Rabbani H, Morett A, Soderstrom K, Levitskaya J, Kiessling R. 2002. IFN-gamma protects short-term ovarian carcinoma cell lines from CTL lysis via a CD94/NKG2A-dependent mechanism. J Clin Invest 110: 1515-23
56. Palmisano GL, Contaldi E, Morabito A, Gargaglione V, Ferrara GB, Pistillo MP. 2005. HLA-E surface expression is independent of the availability of HLA class I signal sequence-derived peptides in human tumor cell lines. Hum Immunol 66: 1-12
57. Horst D, Verweij MC, Davison AJ, Ressing ME, Wiertz EJ. 2011. Viral evasion of T cell immunity: ancient mechanisms offering new applications. Curr Opin Immunol 23: 96-103
58. Koppers-Lalic D, Reits EA, Ressing ME, Lipinska AD, Abele R, Koch J, Marcondes Rezende M, Miranda P, van Leeuwen D, Bienkowska-Szewczyk K, Mettenleiter TC, Rijswijik FA, Tampe R, Neeffes J, Wiertz EJ. 2005. Varicelloviruses avoid T cell recognition by UL49.5-mediated inactivation of the transporter associated with antigen processing. Proc Natl Acad Sci U S A 102: 5144-9
59. Koppers-Lalic D, Verweij MC, Lipinska AD, Wang Y, Quinten E, Reits EA, Koch J, Loch S, Marcondes Rezende M, Daus F, Bienkowska-Szewczyk K, Osterrieder N, Mettenleiter TC, Heemskerk MH, Tampe R, Neeffes JJ, Chowdhury SI, Ressing ME, Rijswijik FA, Wiertz EJ. 2008. Varicellovirus UL 49.5 proteins differentially affect the function of the transporter associated with antigen processing. J Exp Med 204: e1000080
60. Verweij MC, Koppers-Lalic D, Loch S, Klauschies F, de la Salle H, Quinten E, Lehner PJ, Mulder A, Knittler MR, Tampe R, Koch J, Ressing ME, Wiertz EJ. 2008. The varicellovirus UL49.5 protein blocks the transporter associated with antigen processing (TAP) by inhibiting essential conformational transitions in the 6+6 transmembrane TAP core complex. J Immunol 181: 4894-907
61. Ahn K, Meyer TH, Uebel S, Sempe P, Djabadah H, Yang Y, Peterson PA, Fruh K, Tampe R. 1996. Molecular mechanism and species specificity of TAP inhibition by herpes simplex virus ICP47. EMBO J 15: 3247-55
62. Hill A, Jugovic P, York I, Russ G, Bennink J, Yewdell J, Ploegh H, Johnson D. 1995. Herpes simplex virus turns off the TAP to evade host immunity. Nature 375: 411-5
63. Tomazin R, Hill AB, Jugovic P, York I, van Endert P, Ploegh H, Andrews DW, Johnson DC. 1996. Stable binding of the herpes simplex virus ICP47 protein to the peptide binding site of TAP. EMBO J 15: 3256-66
64. York IA, Roop C, Andrews DW, Riddell SR, Graham FL, Johnson DC. 1994. A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8+ T lymphocytes. Cell 77: 525-35
65. Dugan GE, Hewitt EW. 2008. Structural and Functional Dissection of the Human Cytomegalovirus Immune Evasion Protein US6. J Virol 82: 3271-82
66. Hewitt EW, Gupta SS, Lehner PJ. 2001. The human cytomegalovirus gene product US6 inhibits ATP binding by TAP. EMBO J 20: 387-96
67. Hislop AD, Ressing ME, van Leeuwen D, Pudney VA, Horst D, Koppers-Lalic D, Croft NP, Neeffes JJ, Rickinson AB, Wiertz EJ. 2007. A CD8+ T cell immune evasion protein specific to Epstein-Barr virus and its close relatives in Old World primates. J Exp Med 204: 1863-73
68. Horst D, van Leeuwen D, Croft NP, Garstka MA, Hislop AD, Kremmer E, Rickinson AB, Wiertz EJ, Ressing ME. 2009. Specific targeting of the EBV lytic phase protein BNLF2a to the transporter associated with antigen processing results in impairment of HLA class I-restricted antigen presentation. J Immunol 182: 2313-24
69. Aztharova D, Edwards DM, Hammarlund E, Scholz IG, Horst D,
Wagner MJ, Upton C, Wiertz EJ, Silfka MK, Fruh K. 2009. Cowpox virus inhibits the transporter associated with antigen processing to evade T cell recognition. Cell Host Microbe 6: 433-45
70. Byun M, Verweij MC, Pickup DJ, Wiertz EJ, Hansen TH, Yokoyama WM. 2009. Two mechanistically distinct immune evasion proteins of cowpox virus combine to avoid antiviral CD8 T cells. Cell Host Microbe 6: 422-32
71. Garrido F, Cabrera T, Aptsiauri N. 2010. “Hard” and “soft” lesions underlying the HLA class I alterations in cancer cells: implications for immunotherapy. Int J Cancer 127: 249-56
72. Chang CC, Campoli M, Ferrone S. 2003. HLA class I defects in malignant lesions: what have we learned? The Keio journal of medicine 52: 220-9
73. Jordanova ES, Gorter A, Ayachi O, Prins F, Durrant LG, Kenter GG, Garrido F, Cabrera T, Rodriguez F, Maleno I, Real LM, Ruiz-Cabello F, Valero P, Camacho FM, Garrido F. 2008. HLA class I expression in metastatic melanoma correlates with tumor development during autologous vaccination. Cancer immunology, immunotherapy : CII 57: 709-17
74. Kamarashev J, Ferrone S, Seifert B, Boni R, Nestle FO, Burg G, Dummer R. 2001. TAP1 down-regulation in primary melanoma lesions: an independent marker of poor prognosis. Int J Cancer 95: 23-8
75. Seliger B, Ruiz-Cabello F, Garrido F. 2008. IFN inducibility of major histocompatibility antigens in tumors. Advances in cancer research 101: 249-76
76. Garrido F, Algarra I. 2001. MHC antigens and tumor escape from immune surveillance. Advances in cancer research 83: 117-58
77. Feenstra M, Veltkamp M, van Kuik J, Wiertsema S, Slootweg P, van den Tweel J, de Weger R, Tilanus M. 1999. HLA class I expression and chromosomal deletions at 6p and 15q in head and neck squamous cell carcinomas. Tissue Antigens 54: 235-45
78. Jimenez P, Canton J, Collado A, Cabrera T, Serrano A, Real LM, Garcia A, Ruiz-Cabello F, Garrido F. 1999. Chromosome loss is the most frequent mechanism contributing to HLA haplotype loss in human tumors. International journal of cancer. Journal international du cancer 83: 91-7
79. Jones TD, Carr MD, Eble JN, Wang M, Lopez-Beltran A, Cheng L. 2005. Clonal origin of lymph node metastases in bladder carcinoma. Cancer 104: 1901-10
80. Koopman LA, Corver WE, van der Slid AR, Giphart MJ, Fleuren GJ. 2000. Multiple genetic alterations cause frequent and heterogeneous human histocompatibility leukocyte antigen class I loss in cervical cancer. J Exp Med 191: 961-76
81. Maleno I, Lopez Nevot MA, Cabrera T, Salinero J, Garrido F. 2002. Multiple mechanisms generate HLA class I altered phenotypes in laryngeal carcinomas: high frequency of HLA haplotype loss associated with loss of heterozygosity in chromosome region 6p21. Cancer immunology, immunotherapy : CII 51: 389-96
82. Sasatomi E, Finkelstein SD, Woods JD, Bakker A, Swalsky PA, Luketch JD, Fernando HC, Yousem SA. 2002. Comparison of accumulated allelic loss between primary tumor and lymph node metastasis in stage II non-small cell lung carcinoma: implications for the timing of lymph node metastasis and prognostic value. Cancer research 62: 2681-9
83. Weber JC, Meyer N, Pencreach E, Schneider A, Guerin E, Neuville A, Stemmer C, Brigand C, Bacheller P, Rohr S, Keding E, Meyer C, Guenot D, Oudet P, Jaeck D, Gaub MP. 2007. Allelotyping analyses of synchronous primary and metastasis CIN colon cancers identified different subtypes. International journal of cancer. Journal international du cancer 120: 524-32
84. Hicklin DJ, Marincola FM, Ferrone S. 1999. HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. Molecular medicine today 5: 178-86
85. Cabrera T, Lara E, Romero JM, Maleno I, Real LM, Ruiz-Cabello F, Valero P, Camacho FM, Garrido F. 2007. HLA class I expression in metastatic melanoma correlates with tumor development during autologous vaccination. Cancer immunology, immunotherapy : CII 56: 709-17
86. Carretero R, Romero JM, Ruiz-Cabello F, Maleno I, Rodriguez F, Camacho FM, Real LM, Garrido F, Cabrera T. 2008. Analysis of HLA class I expression in progressing and regressing metastatic melanoma lesions after immunotherapy. Immunogenetics 60: 439-47
87. van der Burg SH, Arens R, Melief CJ. 2011. Immunotherapy for persistent viral infections and associated disease. Trends in immunology 32: 97-103
88. van der Burg SH, Melief CJ. 2011. Therapeutic vaccination against human papilloma virus induced malignancies. Current opinion in immunology 23: 252-7
89. Melief CJ, Toes RE, Medema JP, van der Burg SH, Ossendorp F, Offringa R. 2000. Strategies for immunotherapy of cancer. Advances in immunology 75: 235-82
90. Jager D, Jager E, Knuth A. 2001. Immune responses to tumour antigens: implications for antigen specific immunotherapy of cancer. Journal of clinical pathology 54: 669-74
91. Houbiers JG, Nijman HW, van der Burg SH, Drijfhout JW, Kenemans P, van de Velde CJ, Brand A, Momburg F, Kast WM, Melief CJ. 1993. In vitro induction of human cytotoxic T lymphocyte responses against peptides of mutant and wild-type p53. European journal of immunology 23: 2072-7
92. Vermeij R, Leffers N, van der Burg SH, Melief CJ, Daemen T, Nijman HW. 2011. Immunological and clinical effects of vaccines targeting p53-overexpressing malignancies. Journal of biomedicine & biotechnology 2011: 702146
93. Boon T, Couille PG, Van den Eynde BJ, van der Bruggen P. 2006. Human T cell responses against melanoma. Annual review of immunology 24: 175-208
94. Simpson AJ, Caballero OL, Jungbluth A, Chen YT, Old LJ. 2005. Cancer/testis antigens, gametogenesis and cancer. Nature reviews. Cancer 5: 615-25
95. Guilloux Y, Lucas S, Brichard VG, Van Pei A, Viret C, De Plaen E, Brasseur F, Lethe B, Jotereau F, Boon T. 1996. A peptide recognized by human cytolytic T lymphocytes on HLA-A2 melanomas is encoded by an intron sequence of the N-acetylglucosaminyltransferase V gene. The Journal of experimental medicine 183: 1173-83
96. Moreau-Aubry A, Le Guiner S, Labarriere N, Gesnel MC, Jotereau F, Breathnach R. 2000. A processed pseudogene codes for a new antigen recognized by a CD8(+) T cell clone on melanoma. The Journal of experimental medicine 191: 1617-24
97. Van Den Eynde BJ, Gaugler B, Probst-Kepper M, Michaux L, Devuyst O, Lorge F, Weynants P, Boon T. 1999. A new antigen recognized by cytolytic T lymphocytes on a human kidney tumor results from reverse strand transcription. *The Journal of experimental medicine* 190: 1793-800

98. Bopp T, Radsak M, Schmitt E, Schild H. 2010. New strategies for the manipulation of adaptive immune responses. *Cancer immunology, immunotherapy : CII* 59: 1443-8

99. Maeurer MJ, Gollin SM, Martin D, Swaney W, Bryant J, Castelli C, Robbins P, Parmiani G, Storkus WJ, Lotze MT. 1996. Tumor escape from immune recognition: lethal recurrent melanoma in a patient associated with downregulation of the peptide transporter protein TAP-1 and loss of expression of the immunodominant MART-1/Melan-A antigen. *The Journal of clinical investigation* 98: 1633-41

100. Wei ML, Cresswell P. 1992. HLA-A2 molecules in an antigen-processing mutant cell contain signal sequence-derived peptides. *Nature* 356: 443-6

101. Weinzierl AO, Rudolf D, Hillen N, Tenzer S, van Endert P, Schild H, Rammensee HG, Stevanovic S. 2008. Features of TAP-independent MHC class I ligands revealed by quantitative mass spectrometry. *Eur J Immunol* 38: 1503-10

102. Chambers B, Grufman P, Fredriksson V, Andersson K, Roseboom M, Laban S, Camps M, Wolpert EZ, Wiertz EJ, Offringa R, Ljunggren HG, van Hall T. 2007. Induction of protective CTL immunity against peptide transporter TAP-deficient tumors through dendritic cell vaccination. *Cancer Res* 67: 8450-5

103. van Hall T, Wolpert EZ, van Veelen P, Laban S, van der Veer M, Roseboom M, Bres S, Grufman P, de Ru A, Meiring H, de Jong A, Franken K, Teixeira A, Valentijn R, Drijfhout JW, Koning F, Camps M, Ossendorp F, Karre K, Ljunggren HG, Melief CJ, Offringa R. 2006. Selective cytotoxic T-lymphocyte targeting of tumor immune escape variants. *Nat Med* 12: 417-24

104. Oliveira CC, Querido B, Sluijter M, Derbinski J, van der Burg SH, van Hall T. 2011. Peptide transporter TAP mediates between competing antigen sources generating distinct surface MHC-I peptide repertoires. *European journal of immunology* 41: 3114-24
