Therapeutic targeting of immune escaped cancers

Marijt, K.A.

Chapter 8

General discussion

General discussion

MHC class I (MHC-I) antigen presentation plays a pivotal role in anti-tumor immunity. High surface expression of MHC-I molecules is generally correlated with high CD8 T cell infiltrate and improved overall survival in many cancers, including melanoma, lung carcinoma, and ovarium carcinoma[1-4]. In contrast, partial (>50% of cells) or complete loss of MHC-I surface expression is associated with lower overall survival and primary resistance to multiple types of immunotherapy in many cancer patients[5-8], underscoring the importance of MHC-I presentation for spontaneous and therapy-induced anti-tumor immunity. Expression of additional molecules in the tumor microenvironment (TME), such as PD-L1 and HLA-E inhibitory checkpoints, further shape immune responses. The presence of immune cells and the expression of immune-related genes together determine the 'immune landscape' or 'immune contexture' of cancers, while the local production of interferons (IFN) strongly impacts this environment[9]. Notably, immune contexture also includes infiltrating myeloid cells, such as tumor associated macrophages which also highly express PD-L1 immune suppressive molecules (**chapter 2**). Although MHC-I and PD-L1 are both regulated by the IFN pathway, an in-depth study on immune escape of NSCLC showed that the expression of co-inhibitory markers and the loss of MHC-I expression are two independent mechanisms of immune evasion[10]. This classifies tumors into different "types" depending on their surface expression of MHC-I and PD-L1[10,11]. The differential expression of MHC-I and PD-L1 suggests that immune escape of cancer cells occurs through a multitude of distinct "hard-wired" and "soft-wired" modifications[12-14] and knowing which of the mechanisms underlie immune escape determines which immunotherapeutic strategy has the most potential for clinical success. Here, we discuss several acquired immune resistance mechanisms and possible therapeutic strategies to overcome the resistance for immunotherapies.

Importance of IFN-signaling for T cell immunity

The IFNγ signaling pathway is essential for regulating innate and adaptive immune responses[15]. The absence of IFNγ production or cellular responsiveness sensitizes the host for microbial infection, supporting the importance of the cytokine IFNγ. Of note, IFNγ also plays important roles in inhibiting tumor angiogenesis[16], and directly has cytotoxic and cytostatic effects delaying tumor progression[17]. IFNγ is mainly secreted by activated type-I T cells and NK cells upon recognition of their target cells. IFNγ binds to the IFNγ-receptor, activating the IFNγ signaling pathway by phosphorylation of the JAKs and STATs, nuclear translocation, and binding of STAT1 homodimers onto interferon-gamma activation sequences. This triggers the transcription of many immunoregulatory genes, including the expression of antigen presentation molecules like MHC-I, but also immune -inhibiting receptors such as

PD-L1. Therefore, the exact downstream effects of IFNγ is context dependent and essential for regulating the efficacy of an anti-tumor immune response.

Cancers with high PD-L1/MHC-I expression are likely IFNγ-sensitive tumors. These tumors usually have a preexisting intratumoral T cell population, but are limited in exerting their effector function as a result of high inhibitory checkpoint expression. Patients with such tumors are most likely responders to PD-1/PD-L1 blockade mono-therapy, since alleviation the PD-1 inhibitory signals will reinvigorate preexisting anti-tumor T cell immunity.

However, cancers with defects in the IFNγ signaling pathway or downstream effector genes will not increase surface expression of MHC-I upon IFNγ stimulation, and will theoretically display weaker T cell reactivity to their tumors. As a consequence, reduced clinical response rates to checkpoint blockade therapies are indeed observed in this category of patients[6]. Moreover, two studies implicated the crucial role of proficient IFNγ signaling in melanoma patients who acquired resistance to PD-1 or CTLA-4 blockade therapies[18,19], and in patients who never responded to therapy[20]. Loss-of-function mutations in the IFNγ-receptor, and Jak1/ Jak2 caused the lack of response in these patients. Alternatively to these hard-wired defects, *in vivo* CRISPR-Cas9 studies have identified APLN2, PTPN2, and LNK as negative modulators of the IFNγ signaling, also limiting the efficacy of immune responses[21-23]. For patients with IFNγ-insensitive tumors, restoring the IFNγ signaling would be essential in order to fully benefit from immunotherapy, including checkpoint blockade therapies. Recently, using MEK inhibitors to increase IFNγ-sensitivity of cancer cells received considerable attention for immune combination therapies[24-26]. Inhibition of MEK signaling results in downregulation of LNK expression, increases the IFNγ signaling, and restores MHC-I presentation[23,25,26]. Similarly, inhibition of EGFR, upstream of MEK, also results in increased sensitivity to IFNγ and higher levels of MHC-I after IFNγ stimulation[27,28]. However, by enhancing the IFNγ signaling, it concurrently increases the expression of the inhibitory immune checkpoint receptor PD-L1, still limiting an effective immune response[24]. Therefore, IFNγ amplifying therapeutics, like EGFR and MEK inhibitors, need to be combined with immune checkpoint blockade therapy to become optimally effective as treatment for IFNγ-insensitive cancers, as suggested by several pre-clinical[24,26,29] and clinical studies[30].

PI3K inhibitors for combination immunotherapy

Cancer cells deprived from oxygen and glucose similarly lose their ability to perceive an active IFNγ response, resulting in low MHC-I and PD-L1 expression. These metabolically stressed cancers can therefore also be classified as IFNγ-insensitive, MHC-I^{low} tumors (**chapter 3**) and are also likely to benefit from therapies that aim to increase MHC-I presentation in combination with checkpoint blockade therapy. We experimentally showed that inhibi-

tion of PI3K signaling restored IFNγ signaling, increased MHC-I presentation, and improved T cell recognition *in vitro* of cancer cells in nutrient-deficient as well as nutrient-proficient conditions. Of note, although PI3K and MEK are downstream of EGFR signaling, they are part of two different pathways and execute distinct effector functions[31]. Therapeutic inhibition of PI3K signaling in combination with immunotherapy could therefore be an interesting new strategy to improve anti-tumor responses where IFNγ sensitivity is abrogated.

PI3K signaling is important for cancer cells, but is also essential for homeostasis of immune cells. The PI3K family can be divided into three classes, namely class I, II and III[32]. In our studies we used LY294002 and wortmannin (**chapter 3**), which are pan-PI3K inhibitors, inhibiting all classes of the PI3K family. It is important to determine which PI3K family is responsible for the observed MHC-I effects, since inhibition of different PI3K classes has differential effects on immune cells and therefore also may affect the therapeutic strategy. Several studies suggested that class I PI3K isoforms directly affect innate and adaptive immune cells. Selective inhibition of the gamma isoform of PI3K (PI3Kγ), highly expressed in infiltrating tumor associated myeloid cells, restored sensitivity to immune checkpoint blockade as a result of reduced immune suppression by M2-like macrophages in tumors[33,34]. Moreover, inhibition of the PI3K isoform delta (PI3Kδ) has been linked to break regulatory T cell mediated immune tolerance[35]. Interestingly, *ex vivo* inhibition of PI3K and its downstream effector AKT were shown to increase the formation of memory-like CD8 T cells and prolonged anti-tumor responses[36,37]. However, class I PI3K signaling also plays critical roles during development and differentiation of CD4 and CD8 T cells[38]. Inhibiting PI3K isoform gamma or delta during thymopoiesis stops T cell development at the CD4/CD8 double negative stage[39], while PI3Kγ is critical for exerting T cell function in matured T cells[40]. Interestingly, inhibition of AKT or mTOR, which are the main effector molecules of class I PI3K, did not restore MHC-I surface expression on tumor cells under oxygen and glucose deprived conditions (**chapter 3**, data not shown) and perhaps other PI3K classes or other non-canonical PI3K-signaling pathways[41] play important roles at the intersection of the PI3K and STAT1 axis. Class II PI3K was shown to be essential in TCR mediated activation of T cells by modulating the activation of potassium ion channels[42,43]. In cancers, increased expression of class II PI3K (PI3K-C2β) was suggested to be implicated in MEK1/2 activation, which implies that class II PI3K might has a role in MEK1 mediated suppression of MHC-I presentation[23,44,45]. Class III PI3K, or Vps34, plays a critical role in autophagy and endocytosis and is important for in the intracellular trafficking of the IL7-receptor in tumor cells[46]. Mice lacking Vps34 in T cells displayed a decreased number of T cells, suggesting that Vsp34 is an important regulator of naive T cell homeostasis, modulating IL-7Rα trafficking, signaling, and recycling.

Collectively, understanding the distinctive roles of the several isoforms of PI3K is critical for development of effective combination therapies, since modulation of the activity of these PI3K isoforms has differential effects on either tumor cells and host T cells. A delicate balance of single or combinatory specific PI3K inhibitors might be required to ensure improved IFNγ signaling and MHC-I presentation without compromising the effector function of immune cells. Therefore, the use of PI3K-signaling blocking compounds in combination therapies should be carefully evaluated with respect to their effects on T cells.

Immunotherapy using TEIPP antigens

Tumors with minimal MHC-I expression, irrespective of PD-L1 expression, might be a witness of previous ongoing immune response that resulted in acquired immune escape by downmodulation of MHC-I antigen presentation, making the tumor "invisible". Soft-wired loss of TAP function is often observed in cancers that lost their antigen presenting capabilities, especially in lung carcinomas, and melanomas[47,48]. Single agent checkpoint blockade therapy will most likely not result in an effective anti-tumor response, given the fact that the tumor (neo)antigens, that were initially recognized by tumor-reactive TILs, are not presented anymore by the immune escaped MHC-I^{low} cancer cells. Other T-cell based immunotherapeutic strategies, including dendritic cell vaccination, adoptive T cell therapy, and TCR-tg T cell transfers which target conventional tumor antigens are probably also not effective, because in all cases the presentation of their targeted (neo)antigens depends on TAP function[22]. Therefore, identification of alternative tumor antigens specifically presented on MHC-I^{low}, antigen processing-deficient cancer cells is an important objective. We designed a novel strategy to successfully identify those tumor antigens have resulted in the identification of so-called TEIPPs (**chapter 4**). TEIPPs are a subset of non-mutated antigens solely presented on MHC-I^{low} cancers as a result of downregulated TAP1/2 function[49]. TEIPP-presenting cancer cells typically have no TEIPP specific T cell infiltrate given the lack of sufficient MHC-I presentation which limits T cell activation through direct tumor recognition[50]. However, vaccination strategies were capable of igniting strong TEIPP-directed T cell immunity, and a multitude of studies in TEIPP animal models[50,51] motivated the search for human TEIPP antigens (**chapter 5**). After identification of 16 novel HLA-A*0201 binding TEIPP epitopes, we now look forward to therapeutic exploitation of these novel tumor antigens.

Vaccinating strategies with TAP-independent TEIPP peptides could initiate a CD8 T cell response of MHC-I^{low} cancers. Indeed therapeutic intervention via dendritic cell vaccination or therapeutic peptide vaccination resulted in priming of TEIPP-specific T cells and induced efficient homing to TAP-deficient MHC-I^{low} tumors in mouse models[51]. It can be envisioned that this results in increased local IFNγ levels in the tumor microenvironment. As most

TAP-deficient cancers typically have "soft-wired" epigenetic alterations[47,48], the epigenetic inhibition of TAP could be unleashed by this IFNγ and re-activate the antigen processing machinery. This would theoretically result in reduced presentation of TEIPP antigens and re-presentation of conventional tumor antigens, which may restore the efficacy of TILs responding to the conventional antigens. Alternatively, one could also add conventional tumor antigens to such TEIPP vaccines as this would also stimulate the generation of TAP-dependent peptide specific CD8 T cells and could boost the efficacy of the vaccine. This concept has been attractively shown by the group of Dr. Mami-Chouaib, where they combined long peptides of conventional tumor antigens together with a TEIPP peptide. With this combination vaccine they were able to strongly inhibit outgrowth of an immune escaped TAP-deficient lung carcinoma in a mouse model (**chapter 7**)[52]. Combinatorial vaccination therapy with checkpoint blockade therapy, or TCR gene transfers should be explored to fully take advantage of the re-activation of anti-tumor responses toward immune escaped cancers. In **chapter 6** we showed an approach for the development of a TEIPP vaccine as synthetic long peptide (SLP) and also demonstrated that gene transfer of a TEIPP TCR confers their unique specificity.

The exact threshold for decreased TAP function in cancer cells that is required to optimally present TEIPPs remains to be determined. This makes it difficult to predict how many cancer patients are susceptible to TEIPP targeted therapy. Analyses of *in vitro* cultured human cancer cell lines for TEIPP presentation, revealed that two out of six cancers were spontaneously recognized by TEIPP specific T cells. In four other cancer lines, further inactivation of TAP was necessary for proper TEIPP recognition (**chapter 5**). One cancer line, strongly recognized by TEIPP T cells, had relatively low levels of TAP, underscoring the importance of TAP downmodulation for efficient presentation of TEIPP. In a cohort of 135 lung cancer samples, approximately 53% and 32% displayed low to intermediate expression levels of TAP2, respectively[52]. For melanoma, TAP downregulation is observed in 50% of the primary lesions and a remarkable 83% in metastatic lesions[53], suggesting that a significant proportion of cancer patients might be eligible for TEIPP therapy. However, TEIPP antigen expression, proficiency of other components of the MHC-I antigen presentation pathway, and presence of IFNγ in the TME influencing TAP expression are additional factors expected to strongly influence efficient TEIPP presentation. Notably, when both antigen processing and MHC-I expression are reduced, for example in the case of nutrient deprived IFNγ-insensitive B16F10 cancer cells, no increased recognition was observed by TEIPP specific T cells, although the cancer cells were not expressing TAP proteins (data not shown). Interestingly, the nutrient deprived cancer cells were equally well recognized by TEIPP T cells as their proficient counterparts, suggesting that TEIPP peptides were not hampered in their presentation, in contrast to conventional cancer antigens (data not shown). In addition, IFNγ stimulation of cancer cells with hard-wired loss-of-function TAP mutations were bet-

ter recognized by TEIPP specific T cells, probably due to increased MHC-I gene transcription. Therefore, it seems that the ratio of TAP-mediated influx of peptides and availability of empty MHC-I molecules in the ER are important parameters for presentation of TEIPP antigens. Further studies should provide answers on the percentage of cancer patients that are sensitive for TEIPP therapy.

Alternatively, exploration how to induce TEIPP antigen presentation on cancers cells might be an interesting approach for combination immunotherapies. Interesting progress has been made in generating tools to deliver therapeutics specifically to cancer cells. This way, we could specifically target cancer cells with "tools" to temporarily abrogate TAP function in order induce susceptibility to TEIPP-specific T cells. Oncolytic viruses, which particularly replicate in cancer cells, can be armed with genetic products, like TAP-downmodulating proteins or siRNAs[54]. Engineering oncolytic viruses to express siRNA's towards TAP1/2 might induce TAP-deficiency specifically in cancers cells, making those infected cancer cells vulnerable for TEIPP therapy. Alternatively, aptamers conjugated with siRNAs are also used in the clinic to specifically target cancer cells to confer cancer specificity[55]. Currently, together with the group of Dr. Gilboa (Miller school of Medicine, Miami, USA) we are exploring the possibility of temporarily downregulating TAP function via siRNA in tumor cells by tumor directed targeting by nucleolin-binding aptamer conjugates. Our data show that treatment of TAP siRNA-aptamers specifically targets cancers cells, leaving healthy cells untouched. In preclinical mouse tumor models, treatment with TAP siRNA-aptamers results in strong inhibition of outgrowth of tumors and increased survival in mice[56]. The temporal decrease of TAP and thus MHC-I molecules ignited a broad immune response, including TEIPP-specific T cells. Follow up studies in which aptamer-targeted vaccines are included even show more dramatic anti-tumor responses[56]. These studies highlight the benefits of inducing alternative tumor antigens for effective immunotherapies of immune-escaped cancers.

Hard-wired loss of MHC class I presentation

Loss-of-function mutations in the β2m or HLA-A,B,C locus completely abrogates the presentation of MHC-I molecules. Especially in mismatch repair deficient (MMR-d) colon carcinomas as well as lung carcinomas, total loss of MHC-I expression is often observed[14,57]. Several studies illustrated that cancers acquired immune resistance after combination therapies with adoptive T cell transfer and checkpoint blockade, due to loss of HLA-I and/or β2m[7,58,59]. IFNγ stimulation or therapeutics amplifying the IFNγ signaling will not restore MHC-I antigen presentation in these type of cancers, making all previously discussed therapeutic approaches ineffective. However, NK-cells are innate immune cells specialized in targeting HLA-I negative cancers cells[60]. Unlike T cells, NK-cells do not have TCRs to recognize antigens, but are activated when inhibitory receptors are overruled by activating

receptors. The inhibitory receptors of NK cells bind to MHC-I molecules, which explains why NK cells mainly target MHC-I negative cells. One dominant inhibitory checkpoint molecule is NKG2A which is expressed on a majority of NK cells and limits the effector function of NK cells upon binding with non-classical MHC-I molecule HLA-E, overexpressed on most tumor cells[61,62]. Monalizumab, an anti-NKG2A blocking antibody, enhances NK cell activity against multiple tumor cells and is currently tested in several phase II clinical trials in combination with anti-EGFR (cetuximab) or anti-PD-L1 (Durvalumab) for solid cancer patients[62]. Of note, monalizumab not only antagonized the inhibitory signal on NK cells, but also on immunotherapy activated CD8 T cells, improving immune responses through distinct mechanisms[63].

Another exciting development is the manufacturing of TCR gene transfers in NK cells[64]. NK cells that obtained expression of a TCR, exerted the function of T cells phenotypically, metabolically and functionally, while maintaining their NK cell effector function. This way, when these tailored anti-tumor effector cells are used in (TEIPP) neoantigen mediated therapies, it might prevent cancer cells to immune escape by MHC-I loss, due to direct targeting of the NK cell effector function of these TCR-NK-cells.

References

1 Chowell, D. *et al.* Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science* **359**, 582-587, doi:10.1126/science.aao4572 (2018).

2 Shehata, M. *et al.* Human leukocyte antigen class I expression is an independent prognostic factor in advanced ovarian cancer resistant to first-line platinum chemotherapy. *Br J Cancer* **101**, 1321-1328, doi:10.1038/sj.bjc.6605315 (2009).

3 Katz, S. C. *et al.* T cell infiltrate predicts long-term survival following resection of colorectal cancer liver metastases. *Ann Surg Oncol* **16**, 2524-2530, doi:10.1245/s10434-009-0585-3 (2009).

4 Stanton, S. E. & Disis, M. L. Clinical significance of tumor-infiltrating lymphocytes in breast cancer. *J Immunother Cancer* **4**, 59, doi:10.1186/s40425-016-0165-6 (2016).

5 Middha, S. *et al.* Majority of B2M-Mutant and -Deficient Colorectal Carcinomas Achieve Clinical Benefit From Immune Checkpoint Inhibitor Therapy and Are Microsatellite Instability-High. *JCO Precis Oncol* **3**, doi:10.1200/PO.18.00321 (2019).

6 Rodig, S. J. *et al.* MHC proteins confer differential sensitivity to CTLA-4 and PD-1 blockade in untreated metastatic melanoma. *Sci Transl Med* **10**, doi:10.1126/scitranslmed.aar3342 (2018).

7 Sade-Feldman, M. *et al.* Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat Commun* **8**, 1136, doi:10.1038/s41467-017-01062-w (2017).

8 Havel, J. J., Chowell, D. & Chan, T. A. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. *Nat Rev Cancer* **19**, 133-150, doi:10.1038/s41568-019-0116-x (2019).

9 Castro, F., Cardoso, A. P., Goncalves, R. M., Serre, K. & Oliveira, M. J. Interferon-Gamma at the Crossroads of Tumor Immune Surveillance or Evasion. *Front Immunol* **9**, 847, doi:10.3389/fimmu.2018.00847 (2018).

10 Perea, F. *et al.* HLA class I loss and PD-L1 expression in lung cancer: impact on T-cell infiltration and immune escape. *Oncotarget* **9**, 4120-4133, doi:10.18632/oncotarget.23469 (2018).

11 Teng, M. W., Ngiow, S. F., Ribas, A. & Smyth, M. J. Classifying Cancers Based on T-cell Infiltration and PD-L1. *Cancer Res* **75**, 2139-2145, doi:10.1158/0008-5472.CAN-15-0255 (2015).

12 Chatterjee, A. *et al.* Marked Global DNA Hypomethylation Is Associated with Constitutive PD-L1 Expression in Melanoma. *iScience* **4**, 312-325, doi:10.1016/j.isci.2018.05.021 (2018).

13 Shi, Y. Regulatory mechanisms of PD-L1 expression in cancer cells. *Cancer Immunol Immunother* **67**, 1481-1489, doi:10.1007/s00262-018-2226-9 (2018).

14 McGranahan, N. *et al.* Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution. *Cell* **171**, 1259-1271 e1211, doi:10.1016/j.cell.2017.10.001 (2017).

15 Ikeda, H., Old, L. J. & Schreiber, R. D. The roles of IFN gamma in protection against tumor development and cancer immunoediting. *Cytokine Growth Factor Rev* **13**, 95-109 (2002).

16 Hayakawa, Y. *et al.* IFN-gamma-mediated inhibition of tumor angiogenesis by natural killer T-cell ligand, alpha-galactosylceramide. *Blood* **100**, 1728-1733 (2002).

17 Schroder, K., Hertzog, P. J., Ravasi, T. & Hume, D. A. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol* **75**, 163-189, doi:10.1189/jlb.0603252 (2004).

18 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).

19 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).

20 Shin, D. S. *et al.* Primary Resistance to PD-1 Blockade Mediated by JAK1/2 Mutations. *Cancer Discov* **7**, 188-201, doi:10.1158/2159-8290.CD-16-1223 (2017).

21 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).

22 Patel, S. J. *et al.* Identification of essential genes for cancer immunotherapy. *Nature* **548**, 537-542, doi:10.1038/nature23477 (2017).

23 Ding, L. W. *et al.* LNK suppresses interferon signaling in melanoma. *Nat Commun* **10**, 2230, doi:10.1038/s41467-019-09711-y (2019).

24 Kang, S. H. *et al.* Inhibition of MEK with trametinib enhances the efficacy of anti-PD-L1 inhibitor by regulating anti-tumor immunity in head and neck squamous cell carcinoma. *Oncoimmunology* **8**, e1515057, doi:10.1080/2162402X.2018.1515057 (2019).

25 Brea, E. J. *et al.* Kinase Regulation of Human MHC Class I Molecule Expression on Cancer Cells. *Cancer Immunol Res* **4**, 936-947, doi:10.1158/2326-6066.CIR-16-0177 (2016).

26 Choi, H. *et al.* Pulsatile MEK Inhibition Improves Anti-tumor Immunity and T Cell Function in Murine Kras Mutant Lung Cancer. *Cell Rep* **27**, 806-819 e805, doi:10.1016/j.celrep.2019.03.066 (2019).

27 Garrido, G. *et al.* Upregulation of HLA Class I Expression on Tumor Cells by the Anti-EGFR Antibody Nimotuzumab. *Front Pharmacol* **8**, 595, doi:10.3389/fphar.2017.00595 (2017).

28 Watanabe, S. *et al.* Mutational activation of the epidermal growth factor receptor down-regulates major histocompatibility complex class I expression via the extracellular signal-regulated kinase in non-small cell lung cancer. *Cancer Sci* **110**, 52-60, doi:10.1111/cas.13860 (2019).

29 Dushyanthen, S. *et al.* Agonist immunotherapy restores T cell function following MEK inhibition improving efficacy in breast cancer. *Nat Commun* **8**, 606, doi:10.1038/s41467-017-00728-9 (2017).

30 Ribas, A. *et al.* Combined BRAF and MEK inhibition with PD-1 blockade immunotherapy in BRAF-mutant melanoma. *Nat Med* **25**, 936-940, doi:10.1038/s41591-019-0476-5 (2019).

31 Janku, F. Phosphoinositide 3-kinase (PI3K) pathway inhibitors in solid tumors: From laboratory to patients. *Cancer Treat Rev* **59**, 93-101, doi:10.1016/j.ctrv.2017.07.005 (2017).

32 Thorpe, L. M., Yuzugullu, H. & Zhao, J. J. PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nat Rev Cancer* **15**, 7-24, doi:10.1038/nrc3860 (2015).

33 De Henau, O. *et al.* Overcoming resistance to checkpoint blockade therapy by targeting PI3Kgamma in myeloid cells. *Nature* **539**, 443-447, doi:10.1038/nature20554 (2016).

34 Kaneda, M. M. *et al.* PI3Kgamma is a molecular switch that controls immune suppression. *Nature* **539**, 437-442, doi:10.1038/nature19834 (2016).

35 Ali, K. *et al.* Inactivation of PI(3)K p110delta breaks regulatory T-cell-mediated immune tolerance to cancer. *Nature* **510**, 407-411, doi:10.1038/nature13444 (2014).

36 van der Waart, A. B. *et al.* Inhibition of Akt signaling promotes the generation of superior tumor-reactive T cells for adoptive immunotherapy. *Blood* **124**, 3490-3500, doi:10.1182/blood-2014-05-578583 (2014).

37 Bowers, J. S. *et al.* PI3Kdelta Inhibition Enhances the Antitumor Fitness of Adoptively Transferred CD8(+) T Cells. *Front Immunol* **8**, 1221, doi:10.3389/fimmu.2017.01221 (2017).

38 Qin, H. *et al.* The impact of PI3K inhibitors on breast cancer cell and its tumor microenvironment. *PeerJ* **6**, e5092, doi:10.7717/peerj.5092 (2018).

39 Okkenhaug, K. & Vanhaesebroeck, B. PI3K in lymphocyte development, differentiation and activation. *Nat Rev Immunol* **3**, 317-330, doi:10.1038/nri1056 (2003).

40 Finlay, D. K. Regulation of glucose metabolism in T cells: new insight into the role of Phosphoinositide 3-kinases. *Front Immunol* **3**, 247, doi:10.3389/fimmu.2012.00247 (2012).

41 Cao, H. *et al.* Functional role of SGK3 in PI3K/Pten driven liver tumor development. *BMC Cancer* **19**, 343, doi:10.1186/s12885-019-5551-2 (2019).

42 Srivastava, S. *et al.* The class II phosphatidylinositol 3 kinase C2beta is required for the activation of the K+ channel KCa3.1 and CD4 T-cells. *Mol Biol Cell* **20**, 3783-3791, doi:10.1091/mbc.E09-05-0390 (2009).

43 Feske, S., Skolnik, E. Y. & Prakriya, M. Ion channels and transporters in lymphocyte function and immunity. *Nat Rev Immunol* **12**, 532-547, doi:10.1038/nri3233 (2012).

44 Boller, D. *et al.* Targeting PI3KC2beta impairs proliferation and survival in acute leukemia, brain tumours and neuroendocrine tumours. *Anticancer Res* **32**, 3015-3027 (2012).

45 Russo, A. & O'Bryan, J. P. Intersectin 1 is required for neuroblastoma tumorigenesis. *Oncogene* **31**, 4828-4834, doi:10.1038/onc.2011.643 (2012).

46 McLeod, I. X., Zhou, X., Li, Q. J., Wang, F. & He, Y. W. The class III kinase Vps34 promotes T lymphocyte survival through regulating IL-7Ralpha surface expression. *J Immunol* **187**, 5051-5061, doi:10.4049/jimmunol.1100710 (2011).

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

48 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).

49 Marijt, K. A. *et al.* Identification of non-mutated neoantigens presented by TAP-deficient tumors. *J Exp Med* **215**, 2325-2337, doi:10.1084/jem.20180577 (2018).

50 Doorduijn, E. M. *et al.* T cells specific for a TAP-independent self-peptide remain naive in tumor-bearing mice and are fully exploitable for therapy. *Oncoimmunology* **7**, e1382793, doi:10.1080/2162402X.2017.1382793 (2018).

51 Doorduijn, E. M. *et al.* TAP-independent self-peptides enhance T cell recognition of immune-escaped tumors. *J Clin Invest* **126**, 784-794, doi:10.1172/JCI83671 (2016).

52 Durgeau, A. *et al.* Human preprocalcitonin self-antigen generates TAP-dependent and -independent epitopes triggering optimised T-cell responses toward immune-escaped tumours. *Nat Commun* **9**, 5097, doi:10.1038/s41467-018-07603-1 (2018).

53 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).

54 Anesti, A. M., Simpson, G. R., Price, T., Pandha, H. S. & Coffin, R. S. Expression of RNA interference triggers from an oncolytic herpes simplex virus results in specific silencing in tumour cells in vitro and tumours in vivo. *BMC Cancer* **10**, 486, doi:10.1186/1471-2407-10-486 (2010).

55 Yoon, S. & Rossi, J. J. Emerging cancer-specific therapeutic aptamers. *Curr Opin Oncol* **29**, 366-374, doi:10.1097/CCO.0000000000000389 (2017).

56 Greta Garrido, B. S., Ailem Rabasa, Agata Levay, Francesca D'Eramo, Alexey Berezhnoy, Shrey Modi, Tal Gefen, Koen Marijt, Elien Doorduijn, Vikas Dudeja, Thorbald van Hall, Eli Gilboa Tumor-targeted silencing of the peptide transporter TAP induces potent antitumor immunity. *Nat Commun* (2019).

57 Ijsselsteijn, M. E. *et al.* Revisiting immune escape in colorectal cancer in the era of immunotherapy. *Br J Cancer* **120**, 815-818, doi:10.1038/s41416-019-0421-x (2019).

58 Paulson, K. G. *et al.* Acquired cancer resistance to combination immunotherapy from transcriptional loss of class I HLA. *Nat Commun* **9**, 3868, doi:10.1038/s41467-018-06300-3 (2018).

59 Gettinger, S. *et al.* Impaired HLA Class I Antigen Processing and Presentation as a Mechanism of Acquired Resistance to Immune Checkpoint Inhibitors in Lung Cancer. *Cancer Discov* **7**, 1420-1435, doi:10.1158/2159-8290.CD-17-0593 (2017).

60 Caligiuri, M. A. Human natural killer cells. *Blood* **112**, 461-469, doi:10.1182/blood-2007-09-077438 (2008).

61 Kaiser, B. K., Pizarro, J. C., Kerns, J. & Strong, R. K. Structural basis for NKG2A/CD94 recognition of HLA-E. *Proc Natl Acad Sci U S A* **105**, 6696-6701, doi:10.1073/pnas.0802736105 (2008).

62 Andre, P. *et al.* Anti-NKG2A mAb Is a Checkpoint Inhibitor that Promotes Anti-tumor Immunity by Unleashing Both T and NK Cells. *Cell* **175**, 1731-1743 e1713, doi:10.1016/j.cell.2018.10.014 (2018).

63 van Montfoort, N. *et al.* NKG2A Blockade Potentiates CD8 T Cell Immunity Induced by Cancer Vaccines. *Cell* **175**, 1744-1755 e1715, doi:10.1016/j.cell.2018.10.028 (2018).

64 Mensali, N. *et al.* NK cells specifically TCR-dressed to kill cancer cells. *EBioMedicine* **40**, 106-117, doi:10.1016/j.ebiom.2019.01.031 (2019).