Strategies to Improve Cancer Immune Checkpoint Inhibitors Efficacy, other than Abscopal Effect: A Systematic Review

Vito Longo[1]*, Oronzo Brunetti[2]*, Amalia Azzariti[3], Domenico Galetta[1], Patrizia Nardulli[4], Francesco Leonetti[5]° & Nicola Silvestris[6]°

[1]Medical Thoracic Oncology Unit - IRCCS Istituto Tumori "Giovanni Paolo II", Bari

[2]Medical Oncology Unit – Hospital of Barletta

[3]Experimental Pharmacology Laboratory - IRCCS Istituto Tumori "Giovanni Paolo II"

[4]Pharmacy Unit - IRCCS Istituto Tumori "Giovanni Paolo II"

[5]Dipartimento di Farmacia-Scienze del Farmaco - University of Bari

[6]Scientific Direction - IRCCS Istituto Tumori Giovanni Paolo II

All in Italy

*Co-first authors

°Co-last authors

Corresponding author

Nicola Silvestris, MD

Scientific Direction

Cancer Institute "Giovanni Paolo II"

Viale Orazio Flacco, 65

70124 – Bari

Italy

E.mail: n.silvestris@oncologico.bari.it

Abstract

Despite the impact of immune checkpoint inhibitors on malignancies treatment is unprecedented, a lack of response to these molecules is observed in several cases. Differently from melanoma and non-small cell lung cancer, where the use of immune checkpoint inhibitors results in a high efficacy, the response rate in other tumors, such as gastrointestinal cancers, breast cancer, sarcomas, and part of genitor-urinary cancers remains low. The first strategy evaluated to improve the response rate to immune checkpoint inhibitors is the use of predictive factors for the response as PD-L1 expression, tumor mutational burden, and clinical features. In addition to the identification of the patients with a high sensibility to immune checkpoint inhibitors, another approach currently under intensive investigation is the use of therapeutics in a combinatory manner with immune checkpoint inhibitors to obtain an enhancement of efficacy through the modification of the tumor immune microenvironment. In addition to the abscopal effect induced by radiotherapy, a lot of studies are evaluating several drugs able to improve response rate to immune checkpoint inhibitors, including microbiota modifiers, drugs targeting co-inhibitors receptors, anti-angiogenic therapeutics, small molecules, and oncolytic viruses. In view of the rapid and extensive development of this research field, we conducted a systematic review of the literature identifying which of these drugs are closer to achieving validation in the clinical practice.

Keywords

Immunocheckpoint inhibitors; Chemotherapy; Tirosin kinase inhibitors; Angiogenesis

Introduction

Today, immune checkpoint inhibitors (ICIs) represent a gold standard treatment in the first-line setting of several tumors, including non-small cell lung cancer (NSCLC) [1-3], BRAF wild-type (WT) melanoma [4] and metastatic renal cell carcinoma (mRCC) [5]. Nevertheless, a lack of response to these molecules is observed in several cases [6-8].

Mainly, two strategies are considered to improve the response rate to ICIs. The first is represented by the selection of patients according to specific predictive factors (i.e., PD-L1 expression, tumor mutational burden (TMB), and clinical features). The second strategy has the aim to enhance the efficacy of ICIs, with the abscopal effect induced by radiotherapy representing the most frequently evaluated approach in both pre-clinical and clinical setting. However, in the last few years, several studies were focused on the potential role of molecules as being able to improve response rate to ICIs by modifying tumor immune microenvironment, increasing the number of activated T cell exerting effector functions and decreasing the number of immunosuppressive cells thus turning a cold tumor into a hot one. These drugs include microbiota modifiers, drugs targeting co-inhibitors receptors, anti-angiogenic therapeutics, small molecules, and oncolytic viruses. A systematic review of the literature was conducted, considering only the drug classes which are under evaluation in the clinical setting and as much as they could be considered in the clinical practice in the near future. The research has been conducted considering papers published on Pubmed and data presented to the ASCO and ESMO annual meeting.

Materials and Methods

Search strategy

The search strategy was designed to identify peer-reviewed published studies researching the combination of ICIs and other therapies with the aim to enhance anticancer immunological response. The review covered all countries; no date limit was set in order to ensure that a wide range of articles could be identified. A web-based search of MEDLINE/PubMed library data

published from 2010 to December 2018 has been performed. Additional research was performed on ClinicalTrials.gov. Search terms were generated to encapsulate the effect of ICIs on cancer AND the enhancing of anticancer effect (table 1).

Inclusion/Exclusion criteria

To be eligible, papers had to be written in English, published in a peer-reviewed journal, be original primary research including experimental, observational and qualitative studies.

The relevant outcomes explored were in depth as there was a demonstrated role of increased efficacy of the anti-cancer effect of ICIs when these were administrated in combination with other therapies. The Authors excluded the use of ICIs in combination with radiotherapies or other local or regional treatments.

Study selection

All studies identified through the search process were exported to EndNoteversion X7. Duplicates were removed. Two authors (OB and VL) independently double screened the titles, abstracts and key words with the eligibility criteria. Results were compared and contrasted and full-text records of potentially relevant publications were obtained and screened using the inclusion criteria for the final selection of studies for the systematic review.

A panel of experts provided additional biological and clinical information, helping greatly in clarifying some issues in the absence of clear-cut information from the literature.

The final draft was then submitted to the evaluation of experts and modified according to their suggestion and comments.

Microbiota and ICIs

Microbiota plays a crucial role in the development of host immunity [9]. In several pathologies (i.e., inflammatory bowel disease, diabetes, obesity, atherosclerosis, asthma, and dysmetabolic

syndromes) gut commensals result disrupted in comparison with those of unaffected individuals. [10, 11].

Regarding the relationship between microbiota and ICIs, the administration of a combination of broad-spectrum antibiotics (i.e. ampicillin plus colistin plus streptomycin) as well as imipenem alone compromised antitumor effects of CTLA-4 monoclonal antibody (mAb) as a consequence of microbiota impairment. Moreover, administration of antibiotics in patients treated with anti–PD-1/PD-L1 mAb between 2 months before and 2 months after immunotherapy administration resulted in a worse prognosis [12], implying a critical role of microbiota in the modulation of response to ICIs.

In 2015 Vetizou et al. found that anticancer effects of anti-CTLA-4 mAb depended on distinct Bacteroides (B) specimens. In particular, the Authors demonstrated that T-cell specific response for B. thetaiotaomicron and B. fragilis was associated to the efficacy of anti-CTLA-4 administration in mice inoculated with MCA205 sarcomas, Ret melanoma, and MC38 colon cancer cells. Gut bacterial disruption led to a reduction of anticancer response. This deficiency was overcome by administration of B. fragilis, through immunization with B. fragilis polysaccharides, or by adoptive transfer of B. fragilis–specific T cells. Fecal transplantation from metastatic melanoma patients responsive to anti-CTLA-4 in mice inoculated with cancer cells favored the outcome of these mouse tumor models [13]. Indeed, the Authors recolonized both germ-free and antibiotic-treated mice with bacterial species, finding that B. fragilis, B. thetaiotaomicron, B. cepacia, or the combination of B. fragilis and B. cepacia could restore the anti-CTLA-4 mAb effects. Oral combination of B. fragilis and Burkholderia cepacia could restore the efficacy of CTLA4 blockade in antibiotic-treated animals, without incurring in colitis [13, 14]. These data were confirmed in a prospective study considering patients with metastatic melanoma treated with ipilimumab. Gut microbiome enriched with B. phylum was correlated with a low incidence of checkpoint-blockade-induced colitis [15].

In another preclinical study, Sivan et al. compared the growth kinetics of B16.SIY melanoma cells subcutaneously inoculated in two genetically similar C57BL/6 mice from Taconic Farms (TAC) and the Jackson Laboratory (JAX) which contained different gut bacterial communities [16]. TAC mice generated more aggressive tumors than JAX mice. On the contrary, tumor-infiltrating specific CD8+ T cells were more evident in JAX mice than in TAC mice. The Bifidobacterium genus was identified as a driver of tumor response in JAX mice. When both mice were co-housed, all animals showed a JAX phenotype, suggesting that a transferable microbial enhanced immunity response. Moreover, administration of a mixed subspecies of Bifidobacterium impaired tumor growth in TAC mice [16].

Patients with baseline bacterial species with a prevalence of Faecalibacterium genus and other Firmicutes had a significantly longer progression-free survival (PFS) and overall survival (OS) with a more frequent occurrence of colitis than patients with microbiota characterized by the prevalence of B. Moreover, some of these patients reached an OS longer than 18 months [17].

Another study evaluated the therapeutic efficacy of human gut microbiota and its metabolites with different ICIs (i.e., ipilimumab, nivolumab, ipilimumab plus nivolumab, or pembrolizumab). Gut microbiota of responder patients were enriched by B. caccae with high levels of anacardic acid. In particular, bacterial microbiome of patients responsive to the combination of nivolumab plus ipilimumab and pembrolizumab was enriched by Faecalibacterium prausnitzii, B. thetaiotamicron, Holdemania filiformis and Dorea formicogenerans [18].

In 2018, two parallel studies evaluated the role of the microbiome of patients affected by melanoma and treated with anti-PD-1. The first analyzed the oral microbiome of 112 patients without significant differences between responders and non-responders, even if fecal microbiota samples of 30 responders to ICI showed a significant presence of Ruminococcaceae bacteria (p<0.01). In the second study 38 and 4 patients were treated with anti PD1 and anti CTLA4, respectively. A higher presence of Bifidobacterium longum, Collinsella aerofaciens, Enterococcus faecium,

Bifidobacterium adolescentis, Kleibsiella pneumonia, Veillonella parvula, Parabacteriodes merdae, lactobacillus sp.was observed in the gut of responder patients compared with what found in non-responders. Moreover, transplantation of fecal material from responding patients into germ-free mice improved tumor control with greater efficacy than anti-PD-L1 therapy in both studies [19, 20].

Microbiome was evaluated in 249 patients affected by NSCLC, mRCC and urothelial carcinoma (UC) treated with anti-PD-1 [12]. Genomics analysis of patients' stool samples revealed a significant correlation between response to treatment and high presence of Akkermansia muciniphila. The antitumor effects of anti PD-1 blockade were enhanced when fecal microbiota from responding cancer patients was transplanted into germ-free or antibiotic-treated mice. Conversely, fecal transplantation from non-responding patients into germ-free mice did not achieve any result. Oral supplementation with Akkermansia muciniphila in the latter mice restored the efficacy of PD-1.

Sivan et al. demonstrated a higher expression of the class I and II major histocompatibility complex in dentritic cells (DCs) of JAX mice or Bifidobacterium colonized TAC mice. All the above studies showed an influence of microbiota on maturation and activation of DCs (Fig.2). An increase of CD8+/Treg rate was observed in mice transplanted with fecal samples of patients responsive to ICIs. The analysis of tumor infiltrates also revealed the increase of innate effector cells and the reduction of myeloid-derived suppressor cell(MDSC)s [19, 20]. Moreover, administration of Akkermansia muciniphila in anti-PD-1 treated germ free mice was associated with the increase of intra-tumoral helper1 T cells/Tregs rate. Finally, oral administration of A. muciniphila e E. hiraestimulates leads DCs to increase production of IL-12, a cytockine involved in PD-1 inhibition in physiological conditions [12].

In conclusion, there is evidence supporting the relationship between some bacterial species and the enhanced response to ICIs (i.e., Ruminococaceae family of the Firmicutes phylum as Firmicutes prausnitzii) [17-19]. Similarly, other gut microbiome components (i.e., B. and B. phylum) have

been associated with a lack of response to immune checkpoint blockade [17-19]. Data concerning Firmicutes (Roseburia, Streptococcus) [18, 20] and other B. (i.e., Alistipes, Porphyromonas pasteri, and C. aerofaciens) are still not univocal [12, 19, 20]. The differences in the methods used for sample stools and gut microbiome analyses, the databases used for analysis, and populations with both dietary and microbiota differences are responsible for the ambiguity of these data. Clinical trials aimed to define the possible role of microbiote in enhancing the ICIs response are ongoing.

Chemotherapeutics sensitizing tumor to ICIs.

Recently, the combination of immunotherapy plus chemotherapy has been approved for the treatment of both metastatic and locally advanced NSCLC [1-3]. Chemotherapy not only achieves an ulterior efficacy to immunotherapy but it also acts in a synergistic manner in two significant ways: a) induction of immunogenic cell death as part of its independent therapeutic effects; b) disruption strategies used by neoplastic cells to evade immune response. The first process involves the release of tumor antigens and the emission of danger-associated molecular patterns within tumor microenvironment during cell death. At the same time, chemotherapy decreases the number of immunosuppressive cells in the microenvironment including Tregs and MDSCs, increases the number of cytotoxic T lymphocytes (CTLs) and promotes maturation and activation of DCs (Fig.2). In addition, chemotherapeutics modifies the levels of several cytokines, down-regulate immune suppressive cytokines (i.e., transforming growth factor-β (TGF β) and IL10) and up-regulate cytokines promoting tumor immunity (i.e., tumor necrosis factor-α (TNF- α), IL-2, and interferon (INF) – γ) [21].

As far as ICIs are concerned, preclinical models showed that autoctonous tumors that lacked CTLs infiltration were resistant to these agents, while on the contrary the exposure to appropriately selected immunogenic chemotherapeutics induces CTLs tumor infiltration, sensitizing tumor to ICIs. In mouse model of lung adenocarcinoma, refractory to an anti-PD-1 and anti-CTLA-4 mAb combination therapy, the use of oxaliplatin in combination with low-dose cyclophosphamide

increased the lung CTLs/Treg cell ratio sensitizing the tumor to ICIs [22]. Similarly, oxaliplatin increased the amounts of CTLs and activated DCs in a murine colorectal cancer, enhancing the efficacy of a PD-L1 trap [23]. Low-dose of cyclophosphamide combined with an anti-PD1 synergistically induced antigen-specific immunity and the infiltration of CD8+ and CD4+FoxP3⁻ T cells as well as induced the suppression of the CD4+ CD25+ FoxP3+ regulatory T-cell function, thus resulting in the increase of tumor-free survival in a model of cervical cancer [24-25]. According to these studies, 5-fluoruracil (5-FU) increased tumor immunity in a renal cell xenograft mouse model through an increase of CTLs infiltration mediated by the High Mobility Group Box 1 (HMGB1). Interestingly, a 5-FU and anti-PD-L1 combination treatment significantly enhanced the ratio of CTLs and MDSCs compared with 5-FU and anti-PD-L1 single treatments with a longer OS [26].

On the other hand, several chemotherapeutics have been shown to induce an up-regulation of PD-L1 expression, as a possible mechanism of chemotherapy immune suppression. However, the increase of PD-L1 expression may support the synergism between chemotherapy and immunotherapy targeting the PD-L1/PD-1 axis. 5-FU demonstrated up-regulation of PD-L1 in two preclinical studies evaluating colorectal cancer patients [23, 27]. Similarly, the administration of trabectidin induced the INF-γ-dependent PD-L1 expression within tumor in a murine model of ovarian cancer [28]. Others drugs able to up-regulate PD-L1 expression in ovarian cancer models are paclitaxel, carboplatin, cisplatin, gemcitabine, and capecitabine [29]. Interestingly, Peng J et al showed, from a collection of cancer cells from ovarian cancer patients with massive ascites, that the expression of PD-L1 increased 5-fold on day 4 after paclitaxel plus carboplatin therapy and decreased to pre-treatment levels on day 11, demonstrating the reversibility of PD-L1 expression induced by chemotherapy [29]. Finally, the evaluation of 150 specimens of ovarian cancer patients treated with neoadjuvant chemotherapy showed the up-regulation of PD-L1 [30].

Several clinical trials are evaluating the combination of chemotherapy with ICIs, but in the majority of these, chemotherapy and chemoterapeutics are administered concurrently and at full doses. Only few trials are focused on the role of chemotherapeutics as sensitizers for immunotherapy, exploring the optimal dose, or the sequence of administration, while preclinical data have shown that these parameters might affect outcome.

A open label, multi-center, single-arm, Phase Ib/II, evaluated the daily metronomic dose of 50 mg cyclophosphamide without a drug-free break, 10 mg/kg avelumab on day 1 and every other week until progression, and a single fraction of 8 Gy radiotherapy in pretreated head and neck cancer patients, showing absence of unacceptable toxicity [25]. A study concerning metastatic triple negative breast cancer (TNBC) patients investigated induction therapy with various types of chemotherapy [31]. For the induction phase, low doses of chemotherapy were given for 2 weeks: 50 mg daily cyclophosphamide, twice 40 mg/m^2 cisplatin or twice 15 mg doxorubicin. Response rates with chemotherapy appear higher in the cohorts where low-dose chemotherapy was used as induction, compared with nivolumab alone. Conversely, an immunotherapy induction phase may also be useful. An induction phase with durvalumab followed by combination therapy of weekly nab-paclitaxel for 12 weeks followed by four cycles of combination therapy with epirubicin and cyclophosphamide was evaluated in TNBC patients, resulting in a higher pathological CR rate when compared with chemotherapy alone (53.4% versus 44.2%, respectively) [32].

Other trials evaluating the combination of metronomic chemotherapy with ICIs [33] or the impact of chemotherapeutics on TMB [34] are ongoing. Future studies should assess drugs able to induce immunogenic cell death and CTLs tumor microenvironment infiltration, optimizing the integration of ICIs with chemotherapy.

ICIs and antiangiogenic drugs

The vascular network with its specific components (endothelial cells, pericytes, growth factors, and receptors) plays a key role in the regulation of inflammatory response, wound healing, and immune

surveillance. Antigen primed T cells require a healthy endothelium for the trafficking to tissue districts and the cell-to-cell cross-talk during the priming and effector phase of the immune response. The transit of immune cells in the tumor plays a critical role in the outcome of immunotherapeutic strategies, similarly to classical chemotherapeutic drugs. In particular, a normalized endothelium ensures the correct trafficking of T cells to the tumor bed [35]. In fact, tumor angiogenesis contributes to immune tumor escape through the immunosuppressive activity exerted by VEGF, PGE2, IL-10, and tumor hypoxia. In particular, VEGF acts through both the inhibition of lymphocyte adhesion to activated endothelial cells and the systemic effect on immune-regulatory cell function, including the suppression of DCs maturation, the inhibition of T-cell development, and the increase of inhibitory immune cells [36] (Fig.2). Therefore, the possibility of administering ICIs during an anti-angiogenic treatment is being investigated in several cancers according to the hypothesis that vessel normalization induced by anti-angiogenic drugs may enhance immunotherapeutic strategies. On the other hand, the activation of Th1 cells by ICIs blockade increased vessel normalization, suggesting the existence of a mutually regulatory loop [37].

In a phase II study, 46 metastatic melanoma patients were treated in four dosing cohorts of ipilimumab (3 or 10 mg/kg) with four doses at 3-week intervals and then every 12 weeks in combination with bevacizumab (7.5 or 15 mg/kg every 3weeks). Eight partial responses and 22 stable diseases were observed, with a disease-control rate of 67.4% and a median OS of 25.1 months [38]. Bevacizumab has been evaluated also in combination with ICIs targeting the PD-1/PD-L1 axis in a phase II study considering HER2-negative advanced breast cancer patients. The combination of nivolumab, paclitaxel and bevacizumab showed an ORR of 70% [39]. The same combination demonstrated a clinical activity in women with recurrent ovarian cancer which showed an overall confirmed response rate of 21% and a median PFS of 9.4 months [40]. In another study NSCLC patients pre-treated with first-line platinum-based chemotherapy received nivolumab plus bevacizumab as maintenance therapy with 1-yr OS rate of 75% and a manageable toxicity profile

[41]. Recently, the phase III IMpower 150 trial showed no new safety signals of the combination of atezolizumab plus bevacizumab, carboplatin, and taxol in first-line non-squamous NSCLC patients with a median OS of 20.5 months [2]. Interestingly, the first randomized phase III trial of a PD-L1/PD-1 pathway inhibitor combined with bevacizumab in first-line mRCC showed longer PFS for atezolizumab plus bevacizumab compared to sunitinb in PD-L1+ patients [42]. The safety and efficacy of a combined treatment of bevacizumab with atezolizumab was assessed in pre-treated metastatic colon rectal cancer (MCRC) patients, or in oxaliplatin-naïve patients in conjunction with FOLFOX (fluorouracil, folinic acid, leucovorin, and oxaliplatin), with an ORR of 44% in the combination group. In a phase 1a/b study [43] concerning patients with gastric or gastroesophageal junction (G/GEJ), NSCLC, UC, or biliary tract cancer (BTC), the combination of ramucirumab (10 mg/kg) with pembrolizumab (200 mg on the first day of q3w) showed a DCR of 85% with no relevant toxicity [44]. Regarding antiangiogenic TKIs, the combination of nivolumab and either pazopanib or sunitinib has been evaluated in mRCC pre-treated with at least one prior systemic therapy. An ORR of 45% was demonstrated in the nivolumab plus pazopanib arm, compared to 52% in the nivolumab plus sunitinib arm, with a manageable safety profile. These combination approaches might benefit patients with poor prognosis, such as those with a low probability to respond to ICIs monotherapy (i.e., refractory to first-line therapy patients or showing PDL1– negative tumors) [45]. Considering the potential role of antiangiogenenic therapies of changing a cold tumor into a hot one, several trials investigating other combinations of antiangiogenic agents and ICIs are currently ongoing.

Strategies involving other co-inhibitor receptors

The encouraging outcome obtained by the co-inhibitory receptors CTLA-4 and PD-1 prompted the research of additional co-inhibitory molecules. T-cell immunoglobulin and immune-receptor tyrosine-based inhibitory motif domain (TIGIT) is a newly identified co-inhibitory receptor

expressed by Tregs, activated T cells, and natural killer (NK) cells [46]. TIGIT expression is elevated on CD8^{+} TILs and Tregs in a variety of tumors, as well as the expression of its 3 ligands, namely CD155, CD112, and CD11 3 [47]. Moreover, TIGT and PD-1 are co-expressed and up-regulated on TILs. Dual blockade of 2 immune checkpoints enhances function of TILs resulting in a significant tumor rejection, as demonstrated by the combination of anti-CTLA-4 with anti-PD-1/PD-L1. Anti-PD-1 and anti-TIGIT dual therapy significantly improved survival compared to control and monotherapy in a murine glioblastoma (GBM) model. Clinically, TIGIT expression on tumor-infiltrating lymphocytes was shown to be elevated in GBM samples, suggesting that TIGIT pathway may be a valuable therapeutic target [48]. There is an ongoing phase II, randomized, blinded, placebo-controlled study considering MTIG7192A, an anti-TIGIT antibody, in combination with atezolizumab in chemotherapy-naïve NSCLC patients [49].

Lymphocyte activation gene-3 (LAG3), an immune checkpoint up-regulated on activated T cells, Treg, and NK cells in several cancers, is required for the maintenance of Treg suppressive function. LAG3 blocks either by soluble LAG3 immunoglobulin or antibodies have shown efficacy in antitumor response. Similarly to TIGT, LAG3 is coexpressed and upregulated with PD-1 on TILs [50]. According to preclinical data showing a significant augment of activity by dual blockade of LAG3 and PD-1 [51], multiple clinical trials are ongoing with the aim to translate this combination modality into clinical practice.

There is an ongoing phase 1/2a open label trial evaluating BMS-986016, an investigational anti-LAG-3, in combination with nivolumab in patients with advanced melanoma previously treated with anti-PD-1/PD-L1 therapy (n=55. ORR was 12.5% in evaluable patients (n=48). The LAG-3 expression in at least 1% (n=25) of tumor-associated immune cells within the tumor margin was associated with a nearly three-fold improvement of ORRs compared to patients without LAG-3 expression (n=14) (20% and 7.1%, respectively) [52]. LAG525, a humanized IgG4 mAbs able to block the binding of LAG-3 to MHC class II, is under investigation in a phase I/II study in

combination with an anti-PD1 treatment. Common (≥10%) related AEs were fatigue (10%) for LAG525 alone and fatigue (18%), diarrhea (15%), and nausea (12%) in the combination group. LAG525 plus the anti-PD1 drug spartalizumab led to durable RECIST responses (11 PR, 1 CR) in a variety of solid tumors, including mesothelioma (2/8 pts) and triple-negative breast cancer(TNBC) (2/5 pts). In TNBC tumor biopsies, a trend in conversion of immune-cold to immune-activated biomarker profiles was reported [53]. T-cell immunoglobulin and mucin domain-containing 3 (TIM-3) is widely expressed on T helper 1 cells, CD8+ lymphocytes, Treg, DCs, NK cells, and monocytes. Similarly to TIGIT and LAG3, high expression of TIM-3 and PD-1 is observed in the tumor microenvironment, particularly on TILs and Treg, suggesting the possible re-establishing of T cell function through the targeting of TIM-3 and PD-1 [54]. A phase 1 study is evaluating the anti-TIM-3 (T cell immunoglobulin and mucin containing protein-3) antibody TSR-022 as monotherapy and in combination with an anti-PD-1 antibody, in pre-treated patients with advanced solid tumors [55].

Oncolytic virus and ICIs

Oncolytic virus vectors are engineered to have high tumor tropism, maximize cancer-killing effects and minimize the damage to sorrounding normal tissue. Interestingly, these viruses do not only simple lyse tumor cells, but also cause a strong change in the tumor immune microenvironment. In particular, oncolytic viruses transfer genes encoding INF-α, GM-CSF and others cytokines that induce tumor-specific immunity by promoting the maturation and function of DCs. On the other hand, tumor cell lysis induced by oncolytic viruses determines the release of damage molecular patterns (DAMPS) including cell surface proteins, membrane proteins and nucleic acids (Fig 2) [56-57].

Talimogene laherparepvec (T-VEC) replicates within tumors and produces GM-CSF, resulting in a first-in-class intralesional oncolytic immunotherapy approved by the FDA for stage IIIb and IV melanoma. A recent phase II trial comparing ipilimumab plus T-VEC with ipilimumab alone

demonstrated that the ORR in the combination arm was significantly higher than in the monotherapy arm (39% vs 18%; p<0.02) [58]. Distant un-injection sites demonstrated a coadjuvator effect with a reduction in the sizes of visceral lesions in 52% of patients in the combination arm versus only 2% of the patients in the ipilimumab arm. T-Vec has been also tested in melanoma patients in combination with pembrolizumab in a phase Ib study. There were no dose limiting toxicities with an ORR of62% and a CRR of 33% [59]. A phase III trial is ongoing [60]. It is interesting to note that an analysis performed prior to anti-PD1 antibody delivery demonstrated that T-VEC increased the PD-L1 expression and inflammation distant from the injection sites. HF 10, another virus included in the HSV family, in combination with ipilimumab showed in a phase II clinical trial regarding Stage IIIB/IIIC or IV unresectable melanoma a DCR of 68% without disease limiting toxicities [61]. A replication competent oncolytic adenovirus with tumor selectivity namely Tasadenoturev (DNX-2401) resulted to be able to overcome T-cell exhaustion demonstrating a tumor size reduction in a phase I trial for patients with recurrent GBM. A phase II study employing DNX-2401 and pembrolizumab in GBM progressed after initial therapy is currently ongoing [62]. Another group of oncolytic viruses is represented by vaccinia viruses, members of the *Poxviridae* family, which are sitable for transgene insertion. Pexa-Vec targeted tumor associated endothelial cells resulting in vascular distruption and oncolysis [63]. A single dose of intravenous Pexa-Vec demonstrated activation of NK, CD4/CD8 T cells, and antigen presenting cells in surgically treated liver metastases. The combination of Pexa-Vec and nivolumab is under investigation for the treatment of liver tumors [64]. Moreover, the combination of Pexa-Vec with other ICIs is under evaluation in colorectal cancer and other advanced tumors, respectively [65-66]. When speaking about *Coxackie* viruses, CVA 21 is able to increase immune cell infiltration and checkpoint molecules, several clinical trials concerning the combination of CVA 21 with ICIs are ongoing [67]. In particular, in the CAPRA clinical trial, patients receiving multiple intratumoral injections of CVA 21 and pembrolizumab showed an ORR of 73% [68]. Finally, the *reoviruses*, characterized by icosahedral capsid and double-stranded RNA genomes, have been shown to

increase tumor infiltrating cytotoxic T cells CD8+ in a phase Ib concerning GBM patients who underwent debulking neurosurgery [69]. A clinical trial [70] evaluating the use of a reovirus namely pelareorep in combination with pembrolizumab and chemotherapy in patients with relapsed metastatic pancreatic cancers is ongoing.

Small molecule inhibitors and ICIs

Various evidence suggests that small molecule inhibitors could improve tumor–host interactions, enhancing antigen expression and improving immune response against tumor cells [71]. Several small molecules in combination with ICIs have been investigated for the treatment of different cancer histolotypes (table 2).

The first combination of small molecule inhibitors and ICIs have been evaluated in melanoma. In particular, since the administration of BRAFi/MEKi represents a standard treatment of metastatic BRAF^{V600E} melanoma, the possibility that this association would be improved by ICIs has been evaluated. It has been demonstrated that BRAF inhibition is associated with enhanced melanoma antigen expression [72-74]. Moreover, selective BRAF inhibitors induce marked T-cell infiltration in human metastatic melanoma [73], with up-regulation of PD-L1 in the tumor microenvironment [71, 73]. Nevertheless, the benefit of this combination in preclinical models has been modest [75-78]. In particular, in a mouse model of syngeneic BRAF^{V600E} driven melanoma, the combination of dabrafenib and trametinib with pmel-1 adoptive cell transfer showed a complete tumor regression with increased T cell infiltration in tumors and improved *in vivo* cytotoxicity. Single agent dabrafenib increased the number of tumor-associated macrophages and Tregs in tumors that conversely decreased with the addition of trametinib. The combination of BRAFi/MEKi and ICI induced either an increased expression of the melanosomal antigens and MHC or the global immune-related gene up-regulation. Moreover, a combination of dabrafenib and trametinib with anti-PD1 therapy in SM1 tumors led to a superior anti-tumor effect compared to the results obtained with the only small molecules combination [79].

The first phase 1 trial evaluating the role of ipilimumab in combination with vemurafenib was stopped after one month due to liver toxicity [80]. Another phase 1 study evaluated the safety of the combination of dabrafenib, trametinib, and ipilimumab. This study was also stopped due to excessive colon toxicity [81]. A combination of dabrafenib and ipilimumab demonstrated an ORR of 69% in the 26 BRAF-mutated patients with a good safety profile [82]. The KEYNOTE-022, an ongoing phase I/II trial [83], is evaluating the combination of pembrolizumab with dabrafenib and trametinib. Preliminary data on 15 patients enrolled across dose determination and dose confirmation arms showed a safety profile and an ORR of 60% (n= 9 PR, n= 2 SD, n=3 PD) [84]. A phase Ib study is investigating vemurafenib and atezolizumab combination and comparing this combination concurrently or after a run-in period with vemurafenib alone [85]. It was demonstrated that the vemurafenib run-in showed a higher ORR than with concurrent atezolizumab plus vemurafenib start. The combination of atezolizumab, vemurafenib, and cobimetinib in this subset of patients is under investigation [86]. Preliminary results confirmed that this combination has a manageable safety profile with a promising antitumor activity in patients with $BRAF^{V600}$-mutant metastatic melanoma [87].

Also in Gastrointestinal Stromal Tumors (GIST), preclinical studies demonstrated that imatinib in combination with ICIs should improve the immune response. It is well know that this drug induces NK cells activity through DCs in several cancers [88-89]. Furthermore, in an *in vitro* study, imatinib reduced the Treg immunosuppressive function and the FoxP3 expression with the inhibition of phosphorylation of both ZAP70 and LAT, impairing their immunosuppressive function [90]. Moreover, PFS correlated with IFN-γ secretion by NK cells in patients affected by GIST treated with imatinib [91]. In a mouse model of spontaneous GIST, Balachandran et al demonstrated that the immune system substantially contributed to the anti-tumor effects of imatinib. In fact, it activated CD8+ T cells and induced Treg apoptosis in the tumor sample by reducing immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) [92]. In a more recent study, PD-1 was expressed more on T cells in imatinib-treated human GISTs as compared to untreated patients.

Imatinib inhibited the upregulation of PD-L1 through IFNγ in human GIST cell lines. In a GIST mouse model, imatinib downregulated IFNγ related genes and reduced the PD-L1 expression on tumor cells. Moreover, PD-1 or PD-L1 blockade without imatinib achieved no responce in GIST mouse model. On the contrary, association of ICIs and imatinib increased antitumor effects by enhancing cytotoxic T-cell effector function [93].

A current phase I study is evaluating the effect of an ipilimumab and imatinib combination in GIST and other c-Kit positive solid cancers [94]. Preliminary results demonstrated that this combination is safe across multiple tumor types. Nevertheless, low activity with no clear signal for synergy is observed in escalation or GIST expansion cohorts [95].

It is interesting to note that a combination of small molecules and ICIs have been evaluated in a mouse model of oral cancer. In this neoplasia, both activation of PI3K/mTOR and MEK/ERK pathways promoted the immunosuppressive tumor microenvironment [96]. In an immunogenic model of cancer of the oral cavity, rapamycin reduced tumor growth in a CD8-dependent manner [97]. More recently, Moore et al. [98] demonstrated that rapamycin enhanced IFNγ production by peripheral and tumor-infiltrating CD8 T cells in a mouse model of oral cancer. Furthermore, antitumor efficacy was enhanced by CD8 T cell but not by NK cell. Non inflamed tumor models, representing the low responder to immune therapies, did not induce CD8 T cell or NK cell–mediated antitumor immunity when treated with combinations of targeted and ICIs. In other models, antitumor immune responses to PD-L1 mAb treatment were enhanced when treated with mTOR inhibitors. These data suggested that a combination of mTOR inhibitors and ICIs should be evaluated in clinical trials setting.

There are few preclinical studies considering small molecules inhibitors and ICIs combinations in breast cancer patients. In both murine models and breast cancer patients, CDK4/6 inhibition induced anti-tumor immunity through proliferation of Tregs and contributing to anticancer effects [99]. Since cyclin D-CDK4 regulated PD-L1 protein expression, inhibition of CDK4/6 in vivo

increases PD-L1 protein levels through inhibition of cyclin D-CDK4. Combination of CDK4/6 inhibitor and anti-PD-1 immunotherapy enhanced tumor regression and dramatically improved OS rates in mouse breast cancer models [100]. Teo et al. demonstrated that PI3K antagonist and CDK4/6 inhibition significantly increased tumor immunogenicity through generating immunogenic cell death in triple negative breast cancer model. Moreover, this combination significantly increased tumor-infiltrating T-cell activation and cytotoxicity with reduction of immune-suppressive myeloid-derived suppressor cells. Association of immune checkpoints PD-1, CTLA-4 to PI3K antagonist and CDK4/6 inhibition induced complete and durable regressions (>1 year) of breast tumors in *in vivo* models [101].

In the era of precision medicine, several small molecules have been demonstrated to be active in targeting specific pathways leading to apoptosis of cancer cells with impressive results in anti-cancer treatment. In addition, these molecules appear capable of increasing tumor immunogenity through the increase of cancer antigens and the activation of cytotoxic activity of CD8 cells leading to an increased putative activity of ICIs when associated in concomitant or sequential therapeutic schedules.

Conclusions

This systematic review has summarized the current study of the main classes of drugs which improve the activity of the ICIs. The assessment of drugs able to modify tumor immune microenvironment in addition to ICIs is a field of research which is currently undergoing a significant escalation. Despite the encouraging results, none of these molecules has currently entered clinical practice for this specific use. Intriguingly, most of these molecules are characterized by a high level of safety and an already consolidated clinical use for indications other than those considered in this study. These features should allow the possibility of embarking on wider and well-designed studies.

LEGEND Figure 1

Fig. 1 Search strategy with PRISMA flow diagram.

LEGEND Figure 2

Fig. 2 summarizes the mechanisms implicated in improving the efficacy of ICIs: the influence of microbiota on maturation and activation of DCs; the correct trafficking of T cells to the tumor bed due to the normalization of endothelium by anti-angiogenic drugs and the VEGF immunosuppressive acitvity; the impact of chemotherapy on immunosuppressive cells and on the maturation of DCs; release of damaged molecular patterns after oncolytic viruses induce tumor cell lysis.

Conflict of interests

The Authors declare the absence of conflicts of interests.

REFERENCES

1. Gandhi, L.; Rodríguez-Abreu, D.; Gadgeel, S.; Esteban, E.; Felip, E.; De Angelis, F.; Domine, M.; Clingan, P.; Hochmair, M.J.; Powell, S.F.; et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. *N Engl J Med* **2018**, *378*, 2078-2092. doi: 10.1056/NEJMoa1801005.

2. Socinski, M.A.; Jotte, R.M.; Cappuzzo, F.; Orlandi, F.; Stroyakovskiy, D.; Nogami, N.; Rodríguez-Abreu, D.; Moro-Sibilot, D.; Thomas, C.A.; Barlesi, F.; et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *N Engl J Med* **2018**, *378*, 2288-2301. doi: 10.1056/NEJMoa1716948.

3. Antonia, S.J.; Villegas, A.; Daniel, D.; Vicente, D.; Murakami, S.; Hui, R.; Yokoi, T.; Chiappori, A.; Lee, K.H.; de Wit, M.; et al. Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. *N Engl J Med* **2017**, *377*, 1919-1929. doi: 10.1056/NEJMoa1709937.

4. Wolchok, J.D.; Chiarion-Sileni, V.; Gonzalez, R.; Rutkowski, P.; Grob, J.J.; Cowey, C.L.; Lao, C.D.; Wagstaff, J.; Schadendorf, D.; Ferrucci, P.F.;,et al. Overall Survival with Combined Nivolumab and

Ipilimumab in Advanced Melanoma. *N Engl J Med* **2017**, *377*, 1345-1356. doi: 10.1056/NEJMoa1709684.

5. Cella, D.; Grünwald, V.; Escudier, B.; Hammers, H.J.; George, S.; Nathan, P.; Grimm, M.O.; Rini, B.I.; Doan, J.; Ivanescu, C.; et al. Patient-reported outcomes of patients with advanced renal cell carcinoma treated with nivolumab plus ipilimumab versus sunitinib (CheckMate 214): a randomised, phase 3 trial. *Lancet Oncol* **2019**, *20*, 297-310. doi: 10.1016/S1470-2045(18)30778-2.

6. Balar, A.V.; Castellano, D.; O'Donnell, P.H.; Grivas, P.; Vuky, J.; Powles, T.; Plimack, E.R.; Hahn, N.M.; de Wit, R.; Pang, L.; et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. *Lancet Oncol* **2017**, *18*, 1483-1492. doi: 10.1016/S1470-2045(17)30616-2.

7. Borghaei, H.; Paz-Ares, L.; Horn, L.; Spigel, D.R.; Steins, M.; Ready, N.E.; Chow, L.Q.; Vokes, E.E.; Felip, E.; Holgado, E.; et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* **2015**, *373*, 1627-39. doi: 10.1056/NEJMoa1507643.

8. Kanjanapan, Y.; Day, D.; Wang, L.; Al-Sawaihey, H.; Abbas, E.; Namini, A.; Siu, L.L.; Hansen, A.; Razak, A.A.; Spreafico, A.; et al. Hyperprogressive disease in early-phase immunotherapy trials: Clinical predictors and association with immune-related toxicities. *Cancer* **2019**, e/cncr.31999. doi: 10.1002/cncr.31999.

9. Ivanov, I.I.; Honda, K. Intestinal commensal microbes as immune modulators. *Cell Host Microbe* **2012**, *12*, 496-508. doi: 10.1016/j.chom.2012.09.009.

10. Lynch, S.V.; Pedersen, O. The Human Intestinal Microbiome in Health and Disease. *N Engl J Med* **2016**, *375*, 2369-2379.

11. Roy, S.; Trinchieri, G. Microbiota: a key orchestrator of cancer therapy. *Nat Rev Cancer* **2017**, *5*, 271-285. doi: 10.1038/nrc.2017.13.

12. Routy, B.; Le Chatelier, E.; Derosa, L.; Duong, C.P.M.; Alou, M.T.; Daillère, R.; Fluckiger, A.; Messaoudene, M.; Rauber, C.; Roberti, M.P.; et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **2018**, *359*, 91-97. doi: 10.1126/science.aan3706.

13. Vétizou, M.; Pitt, J.M.; Daillère, R.; Lepage, P.; Waldschmitt. N.; Flament, C.; Rusakiewicz, S.; Routy, B.; Roberti, M.P.; Duong, C.P.; et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* **2015**, *350*, 1079-84. doi: 10.1126/science.aad1329.

14. Pitt, J.M.; Vétizou, M.; Gomperts Boneca, I.; Lepage, P.; Chamaillard, M.; Zitvogel, L.; et al. Enhancing the clinical coverage and anticancer efficacy of immune checkpoint blockade through manipulation of the gut microbiota. *Oncoimmunology*, **2016**, *6*, e1132137. doi: 10.1080/2162402X.2015.1132137.

15. Dubin, K.; Callahan, M.K.; Ren, B.; Khanin, R.; Viale, A.; Ling, L.; No, D.; Gobourne, A.; Littmann, E.; Huttenhower, C.; et al. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. *Nat Commun* **2016**, *7*, e10391. doi: 10.1038/ncomms10391.

16. Sivan, A.; Corrales, L.; Hubert, N.; Williams, J.B.; Aquino-Michaels, K.; Earley, Z.M.; Benyamin, F.W.; Lei, Y.M.; Jabri, B.; Alegre, M.L.; et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* **2015**, *350*, 1084-9. doi: 10.1126/science.aac4255.

17. Chaput, N.; Lepage, P.; Coutzac, C.; Soularue, E.; Le Roux, K.; Monot, C.; Boselli, L.; Routier, E.; Cassard, L.; Collins, M.; et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann Oncol* **2017**, *28*, 1368-1379. doi: 10.1093/annonc/mdx108.

18. Frankel, A.E.; Coughlin, L.A.; Kim, J.; Froehlich, T.W.; Xie, Y.; Frenkel, E.P.; Koh, A.Y. Metagenomic Shotgun Sequencing and Unbiased Metabolomic Profiling Identify Specific Human Gut Microbiota and Metabolites Associated with Immune Checkpoint Therapy Efficacy in Melanoma Patients. *Neoplasia* **2017**, *19*, 848-855. doi: 10.1016/j.neo.2017.08.004.

19. Gopalakrishnan, V.; Spencer, C.N.; Nezi, L.; Reuben, A.; Andrews, M.C.; Karpinets, T.V.; Prieto, P.A.; Vicente, D.; Hoffman, K.; Wei, S.C.; et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **2018**, *359*, 97-103. doi: 10.1126/science.aan4236.

20. Matson, V.; Fessler, J.; Bao, R.; Chongsuwat, T.; Zha, Y.; Alegre, M.L.; Luke, J.J.; Gajewski, T.F. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* **2018**, *359*, 104-108. doi: 10.1126/science.aao3290.

21. Emens, L.A.; Middleton, G. The interplay of immunotherapy and chemotherapy: harnessing potential synergies. *Cancer Immunol Res* **2015**, *5*, 436-43. doi: 10.1158/2326-6066.CIR-15-0064.

22. Pfirschke, C.; Engblom, C.; Rickelt, S.; Cortez-Retamozo, V.; Garris, C.; Pucci, F.; Yamazaki, T.; Poirier-Colame, V.; Newton, A.; Redouane, Y.; et al. Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy. *Immunity* **2016**, *44*, 343-54. doi: 10.1016/j.immuni.2015.11.024.

23. Song, W.; Shen, L.; Wang, Y.; Liu. Q.; Goodwin, T.J.; Li, J.; Dorosheva, O.; Liu, T.; Liu, R.; Huang, L. Synergistic and low adverse effect cancer immunotherapy by immunogenic chemotherapy and locally expressed PD-L1 trap. *Nat Commun* **2018**, *9*, 2237. doi: 10.1038/s41467-018-04605-x.

24. Mkrtichyan, M.; Najjar, Y.G.; Raulfs, E.C.; Abdalla, M.Y.; Samara, R.; Rotem-Yehudar, R.; Cook, L.; Khleif, S.N. Anti-PD-1 synergizes with cyclophosphamide to induce potent anti-tumor vaccine effects through novel mechanisms. *Eur J Immunol* **2011**, *41*, 2977-86. doi: 10.1002/eji.201141639.

25. Merlano, M.C.; Merlotti, A.M.; Licitra, L.; Denaro, N.; Fea, E.; Galizia, D.; Di Maio, M.; Fruttero, C.; Curcio, P.; Vecchio, S.; et al. Activation of immune responses in patients with relapsed-metastatic head and neck cancer (CONFRONT phase I-II trial): Multimodality immunotherapy with avelumab, short-course radiotherapy, and cyclophosphamide. *Clin Transl Radiat Oncol* **2018**, *12*, 47-52. doi: 10.1016/j.ctro.2018.08.001.

26. Cui, S. Immunogenic Chemotherapy Sensitizes Renal Cancer to Immune Checkpoint Blockade Therapy in Preclinical Models. *Med Sci Monit* **2017**, *23*, 3360-3366. doi: 10.12659/MSM.902426.

27. Van Der Kraak, L.; Goel, G., Ramanan, K.; Kaltenmeier, C.; Zhang, L.; Normolle, D.P.; Freeman, G.J.; Tang, D.; Nason, K.S.; Davison, J.M.; et al. 5-Fluorouracil upregulates cell surface B7-H1 (PD-L1) expression in gastrointestinal cancers. *J Immunother Cancer* **2016**, *4*, 65. doi: 10.1186/s40425-016-0163-8.

28. Guo, Z.; Wang, H.; Meng, F.; Li, J.; Zhang, S. Combined Trabectedin and anti-PD1 antibody produces a synergistic antitumor effect in a murine model of ovarian cancer. *J Transl Med* **2015**, *13*, 247. doi: 10.1186/s12967-015-0613-y.

29. Peng, J.; Hamanishi, J.; Matsumura, N.; Abiko, K.; Murat, K.; Baba, T.; Yamaguchi, K.; Horikawa, N.; Hosoe, Y.; Murphy, S.K.; et. al. Chemotherapy Induces Programmed Cell Death-Ligand 1 Overexpression via the Nuclear Factor-κB to Foster an Immunosuppressive Tumor

Microenvironment in Ovarian Cancer. *Cancer Res* **2015**, *75*, 5034-45. doi: 10.1158/0008-5472.CAN-14-3098.

30. Mesnage, S.J.L.; Auguste, A.; Genestie, C.; Dunant, A.; Pain, E.; Drusch, F.; Gouy, S.; Morice, P.; Bentivegna, E.; Lhomme, C.; et al. Neoadjuvant chemotherapy (NACT) increases immune infiltration and programmed death-ligand 1 (PD-L1) expression in epithelial ovarian cancer (EOC). *Ann Oncol* **2017**, *28*, 651-657. doi: 10.1093/annonc/mdw625.

31. Kok, M.; Voorwerk, L.; Horlings, H.; Sikorska, K.; van der Vijver, K.; Slagter, M.; Warren, S.; Ong, S.; Wiersma, T.; Russell N.; et al. Adaptive phase II randomized trial of nivolumab after induction treatment in triple negative breast cancer (TONIC trial): Final response data stage I and first translational data. *J Clin Oncol* **2018**, *36*, 15_suppl, 1012-1012. doi: 10.1200/JCO.2018.36.15_suppl.1012.

32. Loibl, S.; Untch, M.; Burchardi, N.; Huober, J.B.; Blohmer, J.U.; Grischke, E.M.; Furlanetto, J.; Tesch, H.; Hanusch, C.; Rezai, M.; et al. Randomized phase II neoadjuvant study (GeparNuevo) to investigate the addition of durvalumab to a taxane-anthracycline containing chemotherapy in triple negative breast cancer (TNBC). *J Clin Oncol* **2018**, *36*, 15_suppl, 104-104. doi: 10.1200/JCO.2018.36.15_suppl.104.

33. NCT03585465: Nivolumab in Combination With Metronomic Chemotherapy in Paediatrics Refractory/Relapsing Solid Tumors or Lymphoma. https://clinicaltrials.gov/ct2/show/NCT03585465. (accessed 10.30.18).

34. NCT03683407: Effect of Chemotherapy on TMB in NSCLC. https://clinicaltrials.gov/ct2/show/NCT03683407. (accessed 10.30.18).

35. Lanitis, E.; Irving, M.; Coukos, G. Targeting the tumor vasculature to enhance T cell activity. *Curr Opin Immunol* **2015**, *33*, 55-63. doi: 10.1016/j.coi.2015.01.011.

36. Stockmann, C.; Schadendorf, D.; Klose, R.; Helfrich, I. The impact of the immune system on tumor: angiogenesis and vascular remodeling. *Front Oncol* **2014**, *4*, e69. doi: 10.3389/fonc.2014.00069.

37. Nuti, M.; Zizzari, I.G.; Botticelli, A.; Rughetti, A.; Marchetti, P. The ambitious role of anti angiogenesis molecules: Turning a cold tumor into a hot one. *Cancer Treat Rev* **2018**, *70*, 41-46. doi: 10.1016/j.ctrv.2018.07.016.

38. Hodi, F.S.; Lawrence, D.; Lezcano, C.; Wu, X.; Zhou, J.; Sasada, T.; Zeng, W.; Giobbie-Harder, A.; Atkins, M.B.; Ibrahim, N.; et al. Bevacizumab plus ipilimumab in patients with metastatic melanoma. *Cancer Immunol Res* **2014**, *2*, 632-42. doi: 10.1158/2326-6066.CIR-14-0053.

39. Ozaki, Y.; Matsumoto, K.; Takahashi, M.; Mukohara, T.; Futamura, M.; Masuda, N.; Tsurutani, J.; Yoshimura, K.; Minami, H.; Takano T. Phase II study of a combination therapy of nivolumab, bevacizumab and paclitaxel in patients with HER2-negative metastatic breast cancer as a first-line treatment (WJOG9917B, NEWBEAT trial). *J Clin Oncol* **2018**; *36*, 15_suppl, TPS1110-TPS1110.

40. Liu, J.F.; Herold, C.; Luo, W.; Penson, R.; Horowitz, N.; Konstantinopoulos, P.; Castro, C.; Curtis, J.; Matulonis, U.A.; Cannistra, S.; et al. A phase 2 trial of combination nivolumab and bevacizumab in recurrent ovarian cancer. *Ann Oncol* **2018**, *29*, suppl_8, viii332-viii358. doi:10.1093/annonc/mdy285.

41. Rizvi, N.A.; Antonia, S.J.; Shepherd, F.A.; Chow, L.Q.; Goldman, J.; Shen, Y.; Chen, A.C. ; Gettinger, S. Nivolumab (Anti-PD-1; BMS-936558, ONO-4538) Maintenance as Monotherapy or in Combination With Bevacizumab (BEV) for Non-Small Cell Lung Cancer (NSCLC) Previously Treated With Chemotherapy. *Int J Radiat Oncol Biol Phys* **2014**, *90*, 5, S32.

42. Motzer, R.J.; Powles, T.; Atkins, M.B.; Escudier, B.; McDermott, D.F.; Suarez, C.; Bracarda, S.; Stadler, W.M.; Donskov, F.; Lee, J.L.; et al. IMmotion151: A Randomized Phase III Study of Atezolizumab Plus Bevacizumab vs Sunitinib in Untreated Metastatic Renal Cell Carcinoma (mRCC). *J Clin Oncol* **2018**, *36*, 6, suppl.578. doi: 10.1200/JCO.2018.36.6_suppl.578

43. NCT024243324: A Study of Ramucirumab Plus Pembrolizumab in Participants With Gastric or GEJ Adenocarcinoma, NSCLC, Transitional Cell Carcinoma of the Urothelium, or Biliary Tract Cancer. https://clinicaltrials.gov/ct2/show/NCT02443324. (accessed 10.30.18).

44. Chau, I.; Penel, N.; Arkenau, H.T.; Santana-Davila, R.; Calvo, E.; Soriano, A.O.; Mi, G.; Jin, J.; Ferry, D.; Herbst, R.S.; et al. Safety and antitumor activity of ramucirumab plus pembrolizumab in treatment naïve advanced gastric or gastroesophageal junction (G/GEJ) adenocarcinoma: Preliminary results from a multi-disease phase I study (JVDF). *J Clin Oncol* **2018**, 36, 4, suppl.101. doi: 10.1200/JCO.2018.36.4_suppl.101

45. Amin, A.; Plimack, E.R.; Ernstoff, M.S.; Lewis, L.D.; Bauer, T.M.; McDermott, D.F.; Carducci, M.; Kollmannsberger, C.; Rini, B.I.; Heng, D.Y.C.; et al. Safety and efficacy of nivolumab in combination with sunitinib or pazopanib in advanced or metastatic renal cell carcinoma: the CheckMate 016 study. *J Immunother Cancer* **2018**, *6*, 109. doi: 10.1186/s40425-018-0420-0.

46. Yu, X.; Harden, K.; Gonzalez, L.C.; Francesco, M.; Chiang, E.; Irving, B.; Tom, I.; Ivelja, S.; Refino, C.J.; Clark, H.; et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nat Immunol* **2009**, 10, 48-57. doi: 10.1038/ni.1674.

47. Mahoney, K.M.; Rennert, P.D.; Freeman, G.J.; Combination cancer immunotherapyand new immunomodulatory targets. *Nat Rev Drug Discov* **2015**, *14*, 561-84. doi: 10.1038/nrd4591.

48. Hung, A.L.; Maxwell, R.; Theodros, D.; Belcaid, Z.; Mathios, D.; Luksik, A.S.; Kim, E.; Wu, A.; Xia, Y.; Garzon-Muvdi, T.; et al. TIGIT and PD-1 dual checkpoint blockade enhances antitumor immunity and survival in GBM. *Oncoimmunology* **2018**, *7*, e1466769. doi: 10.1080/2162402X.2018.1466769.

49. NCT03563716: A Study of MTIG7192A in Combination With Atezolizumab in Chemotherapy-Naïve Patients With Locally Advanced or Metastatic Non-Small Cell Lung Cancer. https://clinicaltrials.gov/ct2/show/NCT03563716. (accessed 10.30.18).

50. Melero, I.; Berman, D.M.; Aznar, M.A.; Korman, A.J.; Perez Gracia, J.L.; Haanen, J. Evolving synergistic combinations of targeted immunotherapies to combat cancer. *Nat Rev Cancer* **2015**, 15, 457-72. doi: 10.1038/nrc3973.

51. Woo, S.R.; Turnis, M.E.; Goldberg, M.V.; Bankoti, J.; Selby, M.; Nirschl, C.J.; Bettini, M.L.; Gravano, D.M.; Vogel, P.; Liu, C.L.; et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res* **2012**, *72*, 917-27. doi: 10.1158/0008-5472.CAN-11-1620.

52. Ascierto, P.A.; Bono, P.; Bhatia, S.; Melero, I.; Nyakas, M.S.; Svane, I.; Callahan, M.K.; Gajewski, T.; Gomez-Roca, C.A.; Hodi, F.S.; et al. Initial efficacy of anti-lymphocyte activation gene-3 (anti–LAG-3; BMS-986016) in combination with nivolumab (nivo) in pts with melanoma (MEL)

previously treated with anti–PD-1/PD-L1 therapy. *J Clin Oncol* **2017**, *35*, 15, suppl 9520. doi: 10.1200/JCO.2017.35.15_suppl.9520.

53. Hong, D.S.; Schoffski, P.; Calvo, A.; Sarantopoulos, J.; De Olza, M.O.; Carvajal, R.D.; Prawira, A.; Kyi, C.; Esaki, T.; Akerley, W.L.; et al. Phase I/II study of LAG525 ± spartalizumab (PDR001) in patients (pts) with advanced malignancies. *J Clin Oncol* **2018**, *36*, 15, suppl 3012. doi: 10.1200/JCO.2018.36.15_suppl.3012.

54. Anderson, A.C.; Joller, N.; Kuchroo, V.K. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. *Immunity* **2016**, *44*, 989-1004. doi: 10.1016/j.immuni.2016.05.001.

55. NCT02817633: A Phase 1 Study of TSR-022, an Anti-TIM-3 Monoclonal Antibody, in Patients With Advanced Solid Tumors (AMBER). https://clinicaltrials.gov/ct2/show/NCT02817633. (accessed 10.30.18).

56. Ribas, A.; Dummer, R.; Puzanov, I.; Vander Walde, A.; Andtbacka, R.H.I.; Michielin, O.; Olszanski, A.J.; Malvehy, J.; Cebon, J.; Fernandez, E.; et al. Oncolytic Virotherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. *Cell* **2017**, *170*, 1109-1119. e10. doi: 10.1016/j.cell.2017.08.027.

57. La Rocca, C.J.; Warner, S.G. Oncolytic viruses and checkpoint inhibitors: combination therapy in clinical trials. *Clin Transl Med* **2018**, *7*, 35. doi: 10.1186/s40169-018-0214-5.

58. Chesney, J.; Puzanov, I.; Collichio, F.; Singh, P.; Milhem, M.M.; Glaspy, J.; Hamid, O.; Ross, M.; Friedlander, P.; Garbe, C.; et al. Randomized, Open-Label Phase II Study Evaluating the Efficacy and Safety of Talimogene Laherparepvec in Combination With Ipilimumab Versus Ipilimumab Alone in Patients With Advanced, Unresectable Melanoma. *J Clin Oncol* **2018**; *36*, 1658-1667. doi: 10.1200/JCO.2017.73.7379.

59. Ribas, A.; Dummer, R.; Puzanov, I.; Vander Walde, A.; Andtbacka, R.H.I.; Michielin, O.; Olszanski, A.J.; Malvehy, J.; Cebon, J.; Fernandez, E.; et al. Oncolytic Virotherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. *Cell* **2018**, *174*, 1031-1032. doi: 10.1016/j.cell.2018.07.035.

60. NCT02263508: Pembrolizumab With or Without Talimogene Laherparepvec or Talimogene Laherparepvec Placebo in Unresected Melanoma (KEYNOTE-034). https://clinicaltrials.gov/ct2/show/NCT02263508 (accessed 10.30.18).

61. Andtbacka, R.H.I.; Ross, M.I.; Agarwala, S.S.; Taylor, M.H.; Vetto, J.T.; Neves, R.I.; Daud, A.; Khong, H.T.; Ungerleider, R.S.; Tanaka, M.; et al. Final results of a phase II multicenter trial of HF10, a replication-competent HSV-1 oncolytic virus, and ipilimumab combination treatment in patients with stage IIIB-IV unresectable or metastatic melanoma. *J Clin Oncol* **2017**, *35*, 15, suppl 9510. doi: 10.1200/JCO.2017.35.15_suppl.9510.

62. NCT02798406: Combination Adenovirus + Pembrolizumab to Trigger Immune Virus Effects (CAPTIVE). https://clinicaltrials.gov/ct2/show/NCT02798406 (accessed 10.30.18).

63. Haddad, D. Genetically Engineered Vaccinia Viruses As Agents for Cancer Treatment, Imaging, and Transgene Delivery. *Front Oncol* **2017**, *7*, 96. doi: 10.3389/fonc.2017.00096.

64. Anthoney, A.; Samson, A.; West, E.; Turnbull, S.J.; Scott, K.; Tidswell, E.; Kingston, J.; Johnpulle, M.; Noutch, S.; Bendjama, K.; et al. Single intravenous preoperative administration of the oncolytic virus Pexa-Vec to prime anti-tumor immunity. *J Clin Oncol* **2018**, *36*, 15, suppl 3092. doi: 10.1200/JCO.2018.36.15_suppl.3092

65. NCT03206073: A Phase I/II Study of Pexa-Vec Oncolytic Virus in Combination With Immune Checkpoint Inhibition in Refractory Colorectal Cancer. https://clinicaltrials.gov/ct2/show/NCT03206073 (accessed 10.30.18).

66. NCT02977156: Immunization Strategy With Intra-tumoral Injections of Pexa-Vec With Ipilimumab in Metastatic/Advanced Solid Tumors. (ISI-JX). https://clinicaltrials.gov/ct2/show/NCT02977156 (accessed 10.30.18).

67. NCT02307149: Intratumoral CAVATAK (CVA21) and Ipilimumab in Patients With Advanced Melanoma (VLA-013 MITCI) (MITCI). https://clinicaltrials.gov/ct2/show/NCT02307149 (accessed 10.30.18).

68. Silk, A.W.; Kaufman, H.; Gabrail, N.; Mehnert, J.; Bryan, J.; Norrell, J.; Medina, D.; Bommareddy, P.; Shafren, D.; Grose M.; et al. Phase 1b study of intratumoral Coxsackievirus A21 (CVA 21) and

systemic pembrolizumab in a dvanced melanoma patients: Interim results of the CAPRA clinical trial. *Cancer Res* **2017**, *77*, 13 Suppl CT026. doi: 10.1158/1538-7445.AM2017-CT026

69. Mahalingam, D.; Fountzilas, C.; Moseley, J.L.; Noronha, N.; Cheetham, K.; Dzugalo, A.; Nuovo, G.; Gutierrez, A.; Arora, S.P.; et al. A study of REOLYSIN in combination with pembrolizumab and chemotherapy in patients (pts) with relapsed metastatic adenocarcinoma of the pancreas (MAP). *J Clin Oncol* **2017**, *35*, 15, suppl e15753. doi: 10.1200/JCO.2017.35.15_suppl.e15753

70. NCT02620423: Study of Pembrolizumab With REOLYSIN® and Chemotherapy in Patients With Advanced Pancreatic Adenocarcinoma. https://clinicaltrials.gov/ct2/show/NCT02620423 (accessed 10.30.18).

71. Frederick, D.T.; Piris, A.; Cogdill, A.P.; Cooper, Z.A.; Lezcano, C.; Ferrone, C.R.; Mitra, D.; Boni, A.; Newton, L.P.; Liu, C.; et al. BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin Cancer Res* **2013**, *19*, 1225-31. doi: 10.1158/1078-0432.CCR-12-1630.

72. Comin-Anduix, B.; Chodon, T.; Sazegar, H.; Matsunaga, D.; Mock, S.; Jalil, J.; Escuin-Ordinas, H.; Chmielowski, B.; Koya, R.C.; Ribas, A.; et al. The oncogenic BRAF kinase inhibitor PLX4032/RG7204 does not affect the viability or function of human lymphocytes across a wide range of concentrations. *Clin Cancer Res* **2010**, *16*, 6040–6048. doi: 10.1158/1078-0432.CCR-10-1911.

73. Wilmott, J.S.; Long, G.V.; Howle, J.R.; Haydu, L.E.; Sharma, R.N.; Thompson, J.F.; Kefford, R.F.; Hersey, P.; Scolyer, R.A. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. *Clin Cancer Res* **2012**, *18*, 1386-94. doi: 10.1158/1078-0432.CCR-11-2479.

74. Bradley, S.D.; Chen, Z.; Melendez, B.; Talukder, A.; Khalili, J.S.; Rodriguez-Cruz, T.; Liu, S.; Whittington, M.; Deng, W.; Li, F.; et al., BRAFV600E co-opts a conserved MHC class I internalization pathway to diminish antigen presentation and CD8+ T-cell recognition of melanoma. *Cancer Immunol Res* **2015**, *3*, 602–609. doi: 10.1158/2326-6066.CIR-15-0030.

75. Koya, R.C.; Mok, S.; Otte, N.; Blacketor, K.J.; Comin-Anduix, B.; Tumeh, P.C.; Minasyan, A.; Graham, N.A.; Graeber, T.G.; Chodon, T.; et al. BRAF inhibitor vemurafenib improves the antitumor

activity of adoptive cell immunotherapy. *Cancer res* **2012**, *72*, 3928. doi: 10.1158/0008-5472.CAN-11-2837.

76. Knight, D.A.; Ngiow, S.F.; Li, M.; Parmenter, T.; Mok, S.; Cass, A.; Haynes, N.M.; Kinross, K.; Yagita, H.; Koya, R.C.; et al. Host immunity contributes to the anti-melanoma activity of BRAF inhibitors. *J Clin Invest* **2016**, *126*, 402-3. doi: 10.1172/JCI84828.

77. Hooijkaas, A.; Gadiot, J.; Morrow, M.; Stewart, R.; Schumacher, T.; Blank, C.U. Selective BRAF inhibition decreases tumor-resident lymphocyte frequencies in a mouse model of human melanoma. *Oncoimmunology* **2012**, *1*, 609-617. doi: 10.4161/onci.20226.

78. Cooper, Z.A.; Juneja, V.R.; Sage, P.T.; Frederick, D.T.; Piris, A.; Mitra, D.; Lo, J.A.; Hodi, F.S.; Freeman, G.J.; Bosenberg, M.W.; et al. Response to BRAF inhibition in melanoma is enhanced when combined with immune checkpoint blockade. *Cancer Immunol Res* **2014**, *2*, 643-54. doi: 10.1158/2326-6066.CIR-13-0215.

79. Hu-Lieskovan, S.; Mok, S.; Moreno, B.H.; Tsoi, J.; Faja, L.R.; Goedert, L.; Pinheiro, E.M.; Koya, R.C.; Graeber, T.G.; Comin-Anduix, B.; et al. Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAFV600E melanoma. *Sci Transl Med* **2015**, *7*, 279ra41. doi:10.1126/scitranslmed.aaa4691

80. Ribas, A.; Hodi, F.S.; Callahan, M.; Konto, C.; Wolchok, J. Hepatotoxicity with combination of vemurafenib and ipilimumab. *N Engl J Med* **2013**, *368*, 1365–1366. doi: 10.1056/NEJMc1302338

81. Minor, D.R.; Puzanov, I.; Callahan, M.K.; Hug, B.A.; Hoos, A. Severe gastrointestinal toxicity with administration of trametinib in combination with dabrafenib and ipilimumab. *Pigment Cell Melanoma Res* **2015,** *28*, 611-2. doi: 10.1111/pcmr.12383. Epub 2015 Jun 23.

82. Puzanov, I.; Callahan, M.; Gerald, P.; Linette, G.; Luke, J.J.; Sosmanet, J.A.; Wolchok, J.D; Hamid, O.; Minor, D.R.; Orford, K.W.; et al. Phase 1 study of the BRAF inhibitor dabrafenib (D) with or without the MEK inhibitor trametinib (T) in combination with ipilimumab (Ipi) for V600E/K mutation–positive unresectable or metastatic melanoma (MM). *J Clin Oncol* **2014**, *32*, 15, suppl 2511. doi: 10.1200/jco.2014.32.15_suppl.2511

83. NCT02130466: A Study of the Safety and Efficacy of Pembrolizumab (MK-3475) in Combination With Trametinib and Dabrafenib in Participants With Advanced Melanoma (MK-3475-022/KEYNOTE-022). https://clinicaltrials.gov/ct2/show/NCT02130466 (accessed 10.30.18).

84. Ribas, A.; Hodi, F.; Lawrence, D.; Atkinson, V.; Starodub, A.; Carlino, M.S.; Fisher, R.A.; Long, G.V.; Miller, W.H.; Huang, Y.; et al. Pembrolizumab (pembro) in combination with dabrafenib (D) and trametinib (T) for BRAF-mutant advanced melanoma: phase 1 KEYNOTE-022 study. *J Clin Oncol* **2016**, *34*, 15_suppl, 3014. doi: 10.1200/JCO.2016.34.15_suppl.3014.

85. Nanda, V.G.Y.; Peng, W.; Hwu, P.; Davies, M.A.; Ciliberto, G.; Fattore, L.; Malpicci, D.; Aurisicchio, L.; Ascierto, P.A.; Croce, C.M.; et al. Melanoma and immunotherapy bridge 2015. Naples, Italy. 1–5 December 2015. *J Transl Med* **2016**; *14*, (Suppl 1): 65. doi: 10.1186/s12967-016-0791-2

86. NCT01656642: A Phase 1b Study of Atezolizumab in Combination With BRAFV600-Mutation Positive Metastatic Melanoma. https://clinicaltrials.gov/ct2/show/NCT01656642. (accessed 10.30.18).

87. Sullivan, R.J.; Gonzalez, R.; Lewis, K.D.; Hamid, O.; Infante, J.R.; Patel, M.R.; Hodi, F.S.; Wallin, J.; Pitcher, B.; Cha, E.; et al. Atezolizumab (A) + cobimetinib (C) + vemurafenib (V) in BRAFV600-mutant metastatic melanoma (mel): Updated safety and clinical activity. *J Clin Oncol* **2017**, *35*, 15_suppl, 3063. doi: 10.1200/JCO.2017.35.15_suppl.3063.

88. Borg, C.; Terme, M.; Taïeb, J.; Ménard, C.; Flament, C.; Robert, C.; Maruyama, K.; Wakasugi, H.; Angevin, E.; Thielemans, K.; et al. Novel mode of action of c-kit tyrosine kinase inhibitors leading to NK cell-dependent antitumor effects. *J Clin Invest* **2004**, *114*, 379–388. doi: 10.1172/JCI21102.

89. Van Dongen, M.; Savage, N.D.; Jordanova, E.S.; Briaire-de Bruijn, I.H.; Walburg, K.V.; Ottenhoff, T.H.; Hogendoorn, P.C.; van der Burg, S.H.; Gelderblom, H.; van Hall, T. Anti-inflammatory M2 type macrophages characterize metastasized and tyrosine kinase inhibitor-treated gastrointestinal stromal tumors. *Int J Cancer* **2010**, *127*, 899–909. doi: 10.1002/ijc.25113.

90. Larmonier, N.; Janikashvili, N.; La Casse, C.J.; Larmonier, C.B.; Cantrell, J.; Situ, E.; Lundeen, T.; Bonnotte, B.; Katsanis, E.; et al. Imatinib mesylate inhibits CD4+ CD25+ regulatory T cell activity and enhances active immunotherapy against BCR-ABL- tumors. *J Immunol* **2008**, *181*, 6955-63.

91. Ménard, C.; Blay, J.Y.; Borg, C.; Michiels, S.; Ghiringhelli, F.; Robert, C.; Nonn, C.; Chaput. N.; Taïeb, J.; Delahaye, N.F.; et al. Natural killer cell IFN-gamma levels predict long-term survival with imatinib mesylate therapy in gastrointestinal stromal tumor-bearing patients. *Cancer Res* **2009**, *69*, 3563–69. doi: 10.1158/0008-5472.CAN-08-3807.

92. Balachandran, V.P.; Cavnar, M.J.; Zeng, S.; Bamboat, Z.M.; Ocuin, L.M.; Obaid, H.; Sorenson, E.C.; Popow, R.; Ariyan, C.; Rossi, F.; et al. Imatinib potentiates antitumor T cell responses in gastrointestinal stromal tumor through the inhibition of Ido. *Nat Med* **2011**, *17*, 1094-100. doi: 10.1038/nm.2438.

93. Seifert, A.M.; Zeng, S.; Zhang, J.Q.; Kim, T.S.; Cohen, N.A.; Beckman, M.J.; Medina, B.D.; Maltbaek, J.H.; Loo, J.K.; Crawley, M.H.; et al. PD-1/PD-L1 Blockade Enhances T-cell Activity and Antitumor Efficacy of Imatinib in Gastrointestinal Stromal Tumors. *Clin Cancer Res* **2017**, *23*, 454-465. doi: 10.1158/1078-0432.CCR-16-1163.

94. NCT01738139: Ipilimumab and Imatinib Mesylate in Treating Participants With Metastatic or Unresectable Solid Tumors.https://clinicaltrials.gov/ct2/show/NCT01738139. (accessed 10.30.18).

95. Reilley, M.J.; Bailey, A.; Subbiah, V.; Janku, F.; Naing, A.; Falchook, G.; Karp, D.; Piha-Paul, S.; Tsimberidou, A.; Fu, S.; et al. Phase I clinical trial of combination imatinib and ipilimumab in patients with advanced malignancies. *J Immunother Cancer* **2017**, *5*, 35. doi: 10.1186/s40425-017-0238-1. eCollection 2017.

96. Loukinova, E.; Dong, G.; Enamorado-Ayalya, I.; Thomas, G.R.; Chen, Z.; Schreiber, H.; Van Waes C. Growth regulated oncogene-alpha expression by murine squamous cell carcinoma promotes tumor growth, metastasis, leukocyte infiltration and angiogenesis by a host CXC receptor-2 dependent mechanism. *Oncogene* **2000**, *19*, 3477–86. doi: 10.1038/sj.onc.1203687

97. Cash, H.; Shay, S.; Moree, E.; Cariso, A.; Uppaluri, R.; Van Waes, C.; Allen, C. mTOR and MEK1/2 inhibition differentially modulate tumor growth and the immune microenvironment in syngeneic models of oral cavity cancer. *Oncotarget* **2015**, *6*, 36400–17. doi: 10.18632/oncotarget.5063.

98. Moore, E.C.; Cash, H.A.; Caruso, A.M.; Uppaluri, R.; Hodge, J.W.; Van Waes, C.; Allen, C.T. Enhanced tumor control with combination mTOR and PD-L1 inhibition in syngeneic oral cavity cancers. *Cancer Immunol Res* **2016**, *4*, 611–620. doi:10.1158/2326-6066.CIR-15-0252.

99. Goel, S.; De Cristo, M.J.; Watt, A.C.; Brin Jones, H.; Sceneay, J.; Li, B.B.; Khan, N.; Ubellacker, J.M.; Xie, S.; Metzger-Filho, O.; et al. CDK4/6 inhibition triggers anti-tumour immunity. *Nature* **2017**, *548*, 471-475. doi: 10.1038/nature23465.

100. Zhang, J.; Bu, X.; Wang, H.; Zhu, Y.; Geng, Y.; Nihira, N.T.; Tan, Y.; Ci, Y.; Wu, F.; Dai, X.; et al. Cyclin D-CDK4 kinase destabilizes PD-L1 via cullin 3-SPOP to control cancer immune surveillance. *Nature* **2018**, *553*, 91-95. doi: 10.1038/nature25015.

101. Teo, Z.L.; Versaci, S.; Dushyanthen, S.; Caramia, F.; Savas, P.; Mintoff, C.P.; Zethoven, M.; Virassamy, B.; Luen, S.J..; McArthur, G.A.; et al. Combined CDK4/6 and PI3Kα Inhibition Is Synergistic and Immunogenic in Triple-Negative Breast Cancer. *Cancer Res* **2017**, *77*, 6340-6352. doi: 10.1158/0008-5472.CAN-17-2210.
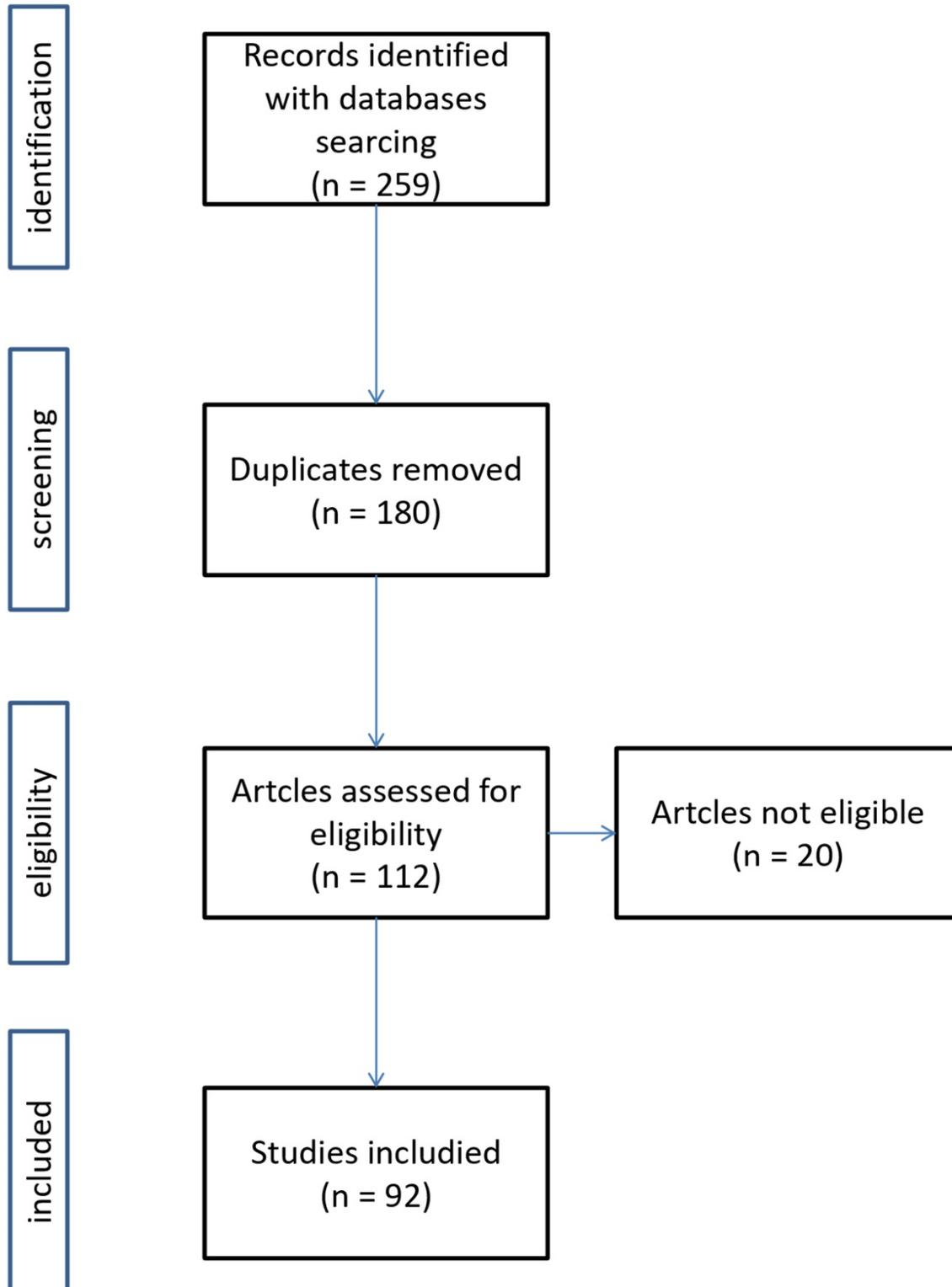
Figure 1

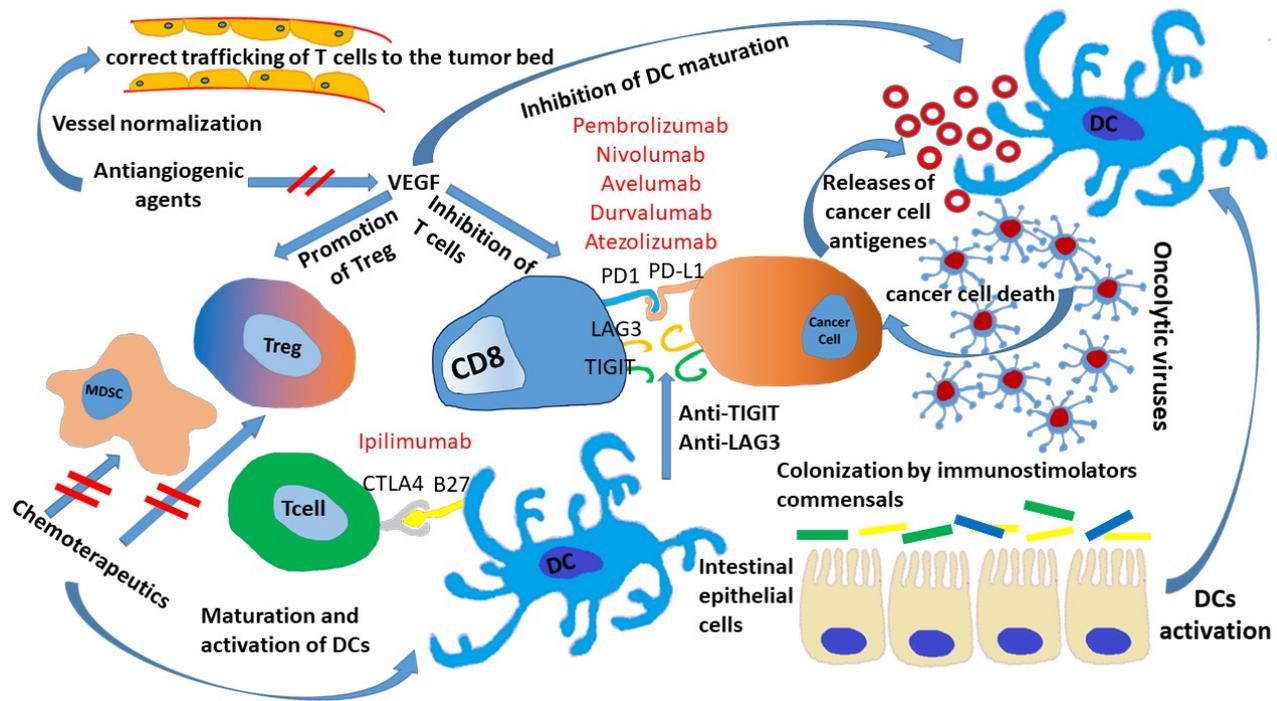## Fig 1. Search strategy with PRISMA flow diagram.

Figure 2

Table 1

Immune therapy	Enhancer
"immunecheckpoint inhibitor" OR "immune therapy" OR "immunocheckpoint blockade"	"microbiote" OR "microbiota" OR "gut microbe" OR "bacteria"
"immunecheckpoint inhibitor" OR "immune therapy" OR "immunocheckpoint blockade"	"chemotherapy" OR" chemotherapeutics" OR "metronomic chemotherapy"
"immunecheckpoint inhibitor" OR "immune therapy" OR "immunochepoint blockade"	"anti-angiogenetic therapies" OR "bevacizumab" OR "nintedanib" OR "Aflibercept" OR "pazopanib" OR "sunitinib"
"immunecheckpoint inhibitor" OR "immune therapy" OR "immunocheckpoint blockade"	"co-inhibitor receptors" OR "TIGIT" OR "LAG3" OR "TIM-3"
"immunecheckpoint inhibitor" OR "immune therapy" OR "immunocheckpoint blockade"	"Oncolytic virus" OR "adenovirus"OR" vaccinia viruses"OR" Coxackie viruses"OR" Reoviruses"
"immunecheckpoint inhibitor" OR "immune therapy" OR "immunocheckpoint blockade"	"small molecules" OR "tyrosine kinase inhibitor" OR "mTOR inhibitor" OR "cycline inhibitor"

Table 2

Small Molecule Enhancer	ICI	Cancer	Study design	Results/Enhancing	Ref.
BRAFi	Not associated	Melanoma	In vitro	BRAF inhibition enhance melanoma antigen expression	Wilmott, 2013
Selective BRAF inhibitors	Not associated	Melanoma	In vitro	Induction of T-cell infiltration into human metastatic melanoma Up-regulation of PD-L1 in tumor microenvironment	Frederick 2013; Wilmott, 2012
Dabrafenib and trametinib	(pmel-1 adoptive cell transfer)	BRAFV600E driven melanoma	In vivo - mouse model	Complete tumor regression with increased T cell infiltration into tumors and improved in vivo cytotoxicity	Cooper, 2014
Dabrafenib and trametinib	anti-PD1	SM1 tumors (melanoma)	In vivo – mouse model	Superior anti-tumor effect compared to the results obtained with the only small molecules combination	Hu-Lieskovan, 2015
Vemurafenib	ipilimumab	Melanoma	Phase 1 trial	Stopped after 1-month due to liver toxicity	Ribas, 2013
Dabrafenib, trametinib	ipilimumab	Melanoma	Phase 1 trial	Stopped for due to excessive colon toxicity	Minor, 2015
Dabrafenib	ipilimumab	BRAF-mutated melanoma	Phase 1 trial	ORR of 69% Good safety profile	Puzanov, l 2014
Dabrafenib and trametinib	pembrolizumab	BRAF-mutated melanoma	KEYNOTE-022, an ongoing phase I/II trial	ORR of 60% (n= 9 PR, n= 2 SD, n=3 PD)	NCT02130466 Ribas, 2016
Vemurafenib (V)	Atezolizumab (A)	Melanoma	Phase Ib trial (V-run in vs concurrent V-A)	Higher ORR was seen with V run-in than with concurrent A + V start	Sullivan, 2016
Vemurafenib, and cobimetinib	atezolizumab	BRAF^{V600}-mutant melanoma	Phase I/II trial	Manageable safety profile and promising antitumor activity	NCT01656642
imatinib	Not associated	GIST	In vitro study	Reduction of Treg immunosuppressive function	Larmonier, 2008
Imatinib	Not associated	GIST	In vivo study	PFS correlated with IFN-γ secretion by NK cells	Ménard, 2009
Imatinib	Not associated	GIST	In vivo – mouse model	Activated CD8+ T cells and induced Treg apoptosis in tumor sample	Vinod,Nat Med. 2011
Imatinib	Anti-PD-1 (RMP1-14) or anti-PD-L1 (10F.9G2)	GIST	In vivo – mouse model	Increased antitumor effects by enhancing cytotoxic T-cell effector function	Seifert, 2017
Imatinib	ipilimumab	GIST and other c-Kit positive solid cancers	Phase 1 trial	Manageable safety profile in multiple tumor types. Low activity with no clear signal for synergy in escalation or GIST expansion cohorts	NCT01738139 Reilley 2017
Rapamycin	Not associated	Oral cancer	In vivo – mouse model	Reduction of tumor growth through CD8-activity	Cash, 2015

Rapamycin	Not associated	Oral cancer	In vivo – mouse model	Enhancing of IFNγ production by peripheral and tumor-infiltrating CD8 T cells	Moore, 2016
Rapamycin	PD-L1 mAb	Oral cancer	In vivo – mouse model	Activation of CD8 T cells in tumor infiltration increased by the addition of rapamycin	Moore, 2016
CDK4/6 inhibitor	Not associated	Breast cancer	In vivo – mouse model/blood sample patients	Anti-tumor immunity through proliferation of Tregs	Goel, 2017
CDK4/6 inhibitor	anti-PD-1	Breast cancer	In vivo – mouse model	Enhancing of tumor regression and dramatically improving of OS	Zhang, 2018
CDK4/6 inhibitor and PI3K antagonist	Anti PD-1 and anti CTLA-4	Triple negative breast cancer	In vivo – mouse model	Inhibition induced complete and durable regressions (>1 year) of breast tumors in in vivo models.	Teo, 2017