Current Trends in the Clinical Development of Combination Therapies with Immune Checkpoint Inhibitors

Authors: Oana Draghiciu, Inka Pawlitzky

Author affiliations: CATO SMS, Walaardt Sacréstraat 401-403, 1117 BM Schiphol, The Netherlands

Summary

- Immune checkpoint inhibitor (ICI) monotherapy holds the promise for durable responses in adequately selected patient subsets and opens the door for novel therapeutic strategies to improve current treatment outcomes
- CATO SMS' analysis of ongoing Phase II and III trials between 2019 and the 1st half of 2020 identified clinical development trends that clearly favor ICIs being assessed primarily as part of combination therapies, with the Top 5 combinations being ICIs plus either targeted therapy, chemotherapy, another ICI, radiotherapy or chemoradiotherapy
- ICI combination therapies remain predominantly evaluated in solid tumors, with the most commonly investigated indications being lung, head and neck, esophageal, melanoma, renal, breast, bladder and gastric cancers
- Next-generation ICIs that target different immune pathways are entering the clinic beyond proof-of-concept trials and several combinations with these next-generation ICIs are currently in clinical development
- CATO SMS provides an in-depth summary of the recent trial landscape for ICI combination therapies with the aim to guide the design and considerations of future clinical trials for novel and tailored therapeutic combinations

Introduction

Developing novel and effective cancer treatment is complex and challenging, defined by an increased heterogeneity in the options for therapeutic modalities. In the last decade, the relevance of cancer immunotherapy using immune checkpoint inhibitors (ICIs) was defined by clinical trial results with ICI monotherapy reporting initial response rates in specific cancers of up to 40% (Cogdill et al, 2017). Despite these promising results, monotherapy of ICIs leads to durable disease control in only 20%-30% of patients and only in specific indications (Hodi et al, 2010; Robert et al, 2015), with patient subsets experiencing primary resistance or disease progression following the initial response (acquired resistance). Research showed that primary resistance is the result of insufficient intratumoral T cell infiltration and anergy of infiltrated T cells due to immune suppressive characteristics of the tumor microenvironment (Spranger et al, 2013; Hugo et al, 2016). Mechanisms of acquired resistance include dysfunctionalities in the antigen maturation process, impaired antigen presentation (Sucker et al, 2014; Zaretsky et al, 2016; Restifo et al, 2016), loss of T-cell function through expression of alternative immune checkpoints (Koyama et al, 2016) and disruption of interferon gamma (IFNγ) signaling (Dunn et al, 2005; Zaretsky et al, 2016).

In this context of resistance, identifying strategies that simultaneously target multiple immune modulating mechanisms are the current focus of therapies in clinical trials. Additionally, strategies that allow to identify and select patients that most likely show a favorable response will improve outcomes of immune therapies.

One of the strategies to select a potentially responsive patient population is the identification and use of biomarkers for predictive and prognostic read-outs. A recently identified biomarker playing a central role in treatment response of solid tumors, such as non-small cell lung cancer

(NSCLC) (Campesato et al, 2015; Rizvi et al, 2015; Carbone et al; 2017), colorectal cancers (Le et al, 2015), urothelial cancers (Balar et al, 2017; Powles et al, 2018), and melanoma (Snyder et al, 2014; Van Allen et al, 2015; Johnson et al, 2016; Eroglu et al, 2018) is the tumor mutational burden (TMB). TMB is the measure of the total amount of somatic coding mutations within tumor cells. An increased TMB results in higher numbers of tumor-specific mutant epitopes functioning as neoantigens, which can be recognized by immune cells to activate a tumor-specific immune response in the setting of ICIs (**Figure 1A**). Use of TMB allows for a

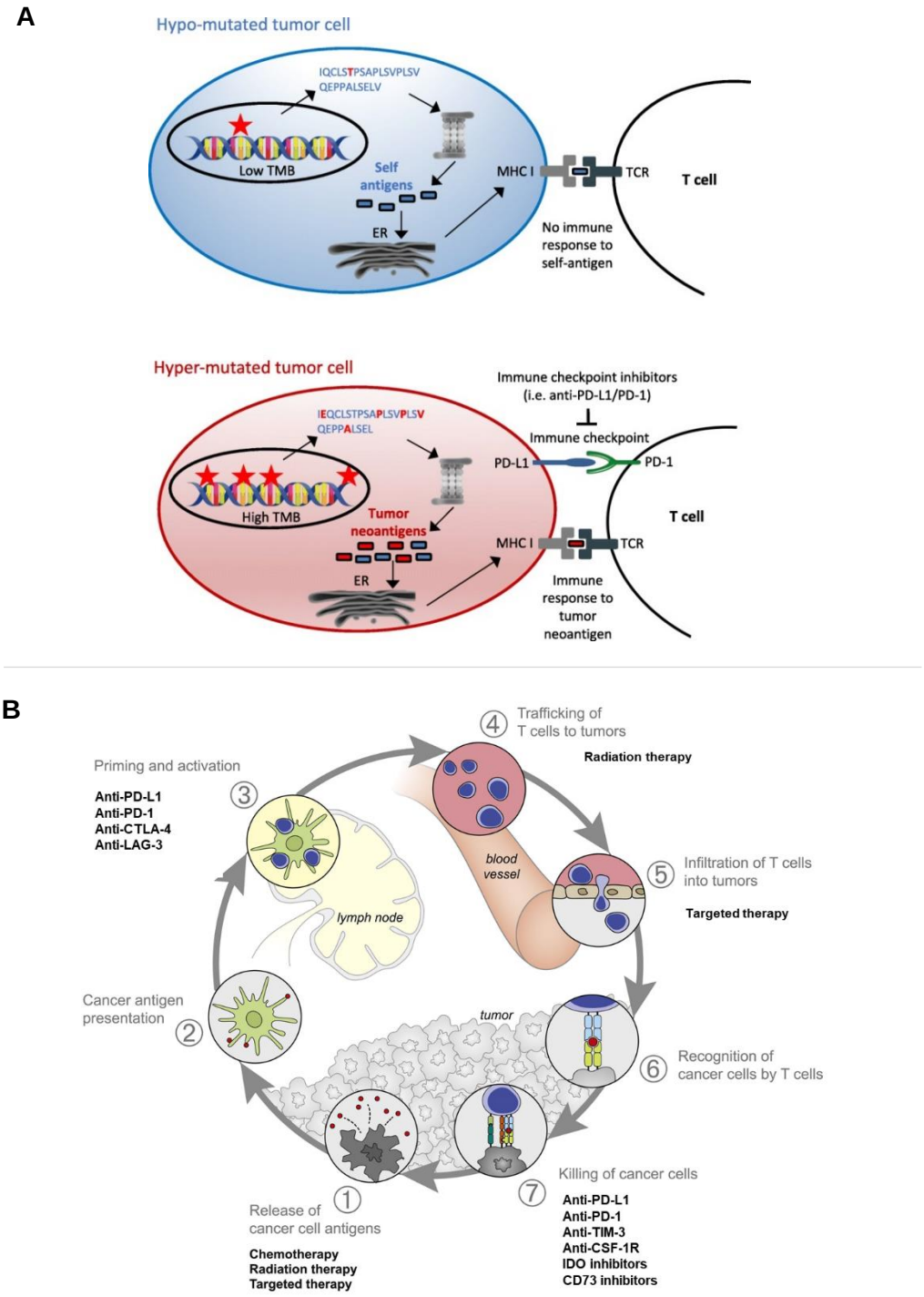


Figure 1 **Strategies for enhancing response to anticancer therapy:** A) Use of TMB as biomarker for adequate selection of therapies (reproduced from *Francello et al,* 2019) and B) Use of ICI combination therapies for simultaneous targeting of several inhibitory factors in the cancer-immunity cycle (modified from *Chen and Melman*, 2013).

tumor agnostic treatment approach which is highlighted by the recent approval of pembrolizumab (anti-programmed cell death protein 1 [PD-1] antibody) for the treatment of TMB-high (TMB-H) tumors by the Food and Drug Administration (FDA).

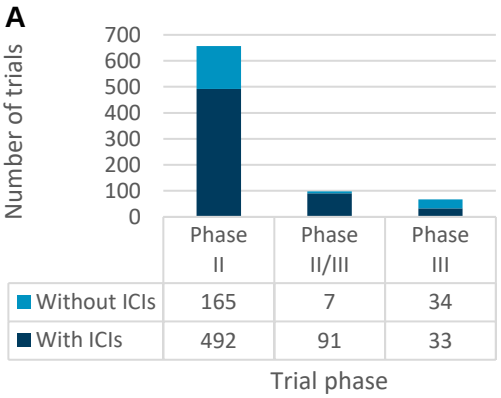
Another investigated strategy to improve response to ICIs is the combination with other immunotherapies or standard therapeutic modalities such as chemotherapy or radiotherapy. ICI combination therapies aim to stimulate several factors of the cancer-immunity cycle (**Figure 1B**), thereby activating multiple immune mechanisms and preventing tumor immune escape. To summarize the progress in the discovery of promising ICI combination therapies between 2014 and 2018, a quantitative and qualitative data analysis was previously performed by CATO SMS. The results of this analysis reported that, from the immuno-oncology clinical trials started between January 2014 and January 2019, 90% assessed ICIs with at least one other therapy in combination (Draghiciu et al, 2019). The top 5 evaluated combination therapies were: ICIs and chemotherapy, two different ICIs, ICIs and radiotherapy, ICIs plus targeted therapies and ICIs plus chemoradiotherapy (Draghiciu et al, 2019).

One aim of the current paper is to assess whether this clinical trial landscape shifted between 2019 and the 1st half of 2020. We focused on recent clinical developments in the field of ICI combination therapies as well as on next-generation ICIs.

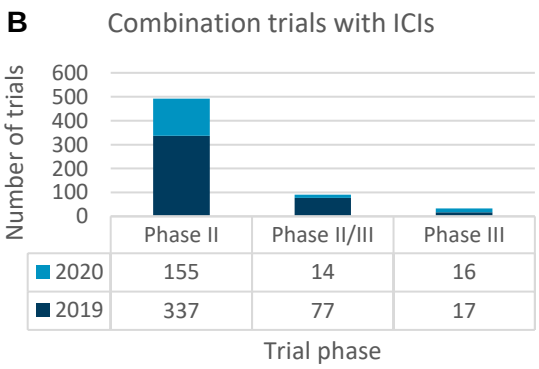
Current Clinical Developments 2019 to 2020

Immuno-oncology trials assessing ICI combination therapies

To elucidate recent developments in the clinical trial landscape assessing ICI combination therapies, the competitive intelligence data platform GlobalData Plc was used to gather and analyze relevant information. The public clinical trial database Clinicaltrials.gov was used as validation and back-up database. All immuno-oncology Phase II, II/III, and III combination trials initiated between January 1st, 2019 and June 30th, 2020, and those assessing ICI combination regimens, were part of the analysis.



The current analysis included a total of 822 immuno-oncology combination trials initiated between 2019 to 2020, of which 75% assessed ICI combination therapies (**Figure 2A**). The majority of trials assessing ICI combination therapies are Phase II (80%, **Figure 2B and C**) One observation is the difference in ongoing Phase III trials between combination trials where ICIs are part of (6%) and combination trials without ICIs (20%). This



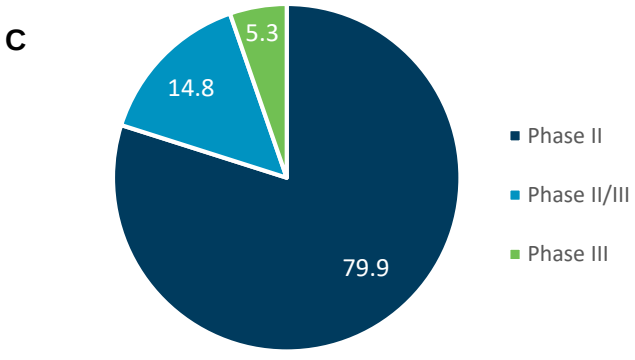


Figure 2 Combination immune-oncology trials initiated between Jan 1st, 2019 and Jun 30th, 2020. Depicted are: the numbers of immuno-oncology combination trials per trial phase (A) and ICI combination trials per trial phase and year (B); the percentages of ICI combination trials per phase (C).

potentially indicates that Phase II trials with ICIs may not meet their endpoints, highlighting that there is a clear need for improved rationales for combination trials with ICIs and for an improved mechanistic understanding to enable biology-driven combination approaches.

Top 5 combination therapies with ICIs

Assessment of the types of combination therapies with ICIs evaluated in the trials that started during the same period revealed that around one-fourth (26%) of the total landscape of ICI combination trials consisted of ICIs plus targeted therapy (**Figure 3A**). This is followed closely by the combination of ICIs plus chemotherapy, assessed in 24% of the analyzed trials (**Figure 3A**). In 11% of the ICI combination trials, the complementary therapy used was a second ICI compound, whereas 7% of trials evaluated the combination with radiotherapy. Approaches using two standard of care therapies, chemotherapy and radiotherapy, plus an ICI (5%) complete the Top 5 combinations assessed in the selected trials (**Figure 3A**). Additionally, the remainder of ICI combination clinical trials (28%) investigate combinations with: a targeted therapy plus a second ICI, with two different targeted therapies, with antiangiogenic compounds alone or plus chemo- or radiotherapy, with cellular therapy or vaccines. Noteworthy, while the identity of the Top 5 combinations with ICIs assessed in clinical trials did not change as compared to the period of 2014-2018 (Draghiciu et al, 2019), the updated analysis highlights an increasing prevalence of targeted therapies being assessed in combination with ICIs starting 2019 (from 5% during 2014-2018 to 26% of combination trials).

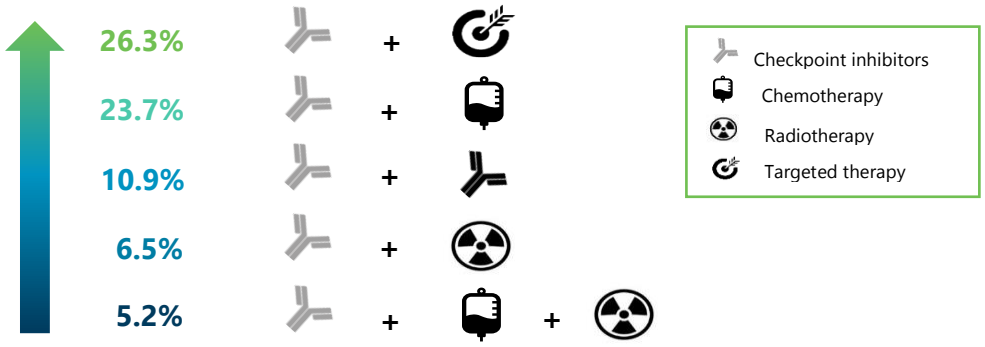
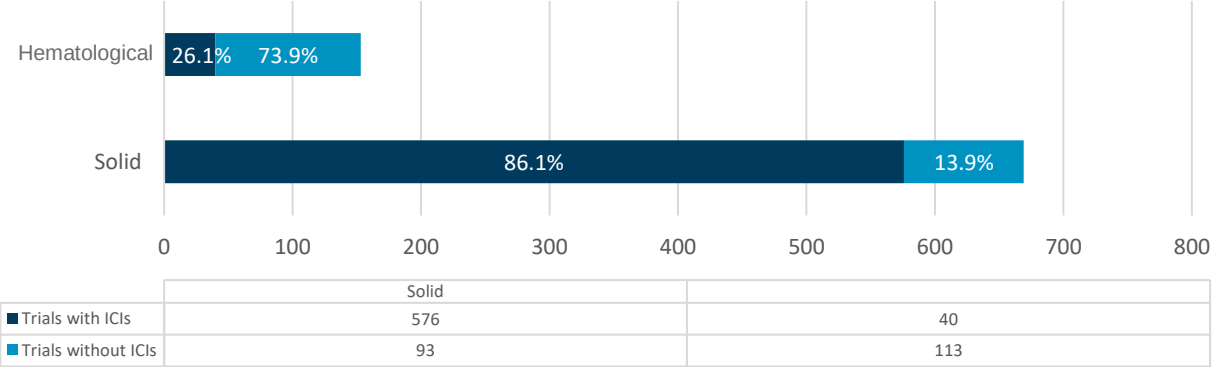


Figure 3A **The top five combination therapies with ICIs.** Extracted from ICI combination therapies investigated in trials initiated between Jan 1st, 2019 and Jun 30th, 2020.

To further understand the development focus of ICI combination regimens in the entire cancer immunotherapy field, we asked whether ICI combination therapies are predominantly investigated in solid or in hematological tumors, or in both. Interestingly, ICI combination therapies are used with a significantly higher percentage in solid versus hematological



	Solid	
Trials with ICIs	576	40
Trials without ICIs	93	113

Figure 3B **The use of immuno-oncology combination therapies with and without ICIs in solid and hematological cancers.** Extracted from ICI combination therapies investigated in trials initiated between Jan 1st, 2019 and Jun 30th, 2020.

malignancies (86% vs 26%%). This situation appears reversed when specifically looking at immuno-oncology combination trials that do not investigate ICIs (14% vs 74%; **Figure 3B**). These opposite results are similar to those reported in our previous analysis for 2014-2018 by CATO SMS (Draghiciu et al, 2019) and highlight a consistent trend regarding the use of ICI combination therapies over > 6 years.

Prominent tumor indications investigated in ICI combination therapy trials

In the current analysis, more than 26 different cancer types were categorized based on their frequency of assessment in ICI combination trials initiated between Jan 1st, 2019 and Jun 30th, 2020. The most frequent investigated cancers, altogether covering 54% of the entire ICI combination trial landscape, were: lung (16%), head and neck (6%), esophageal (6%), melanoma (6%%), renal (5%), breast (5%), bladder (5%) and gastric (5%) cancers (**Figure 4**). The top 10 tumor indications assessed in ICI combination trials are all solid tumors. Lymphomas are the most trialed indication in terms of hematological malignancies in this analysis.

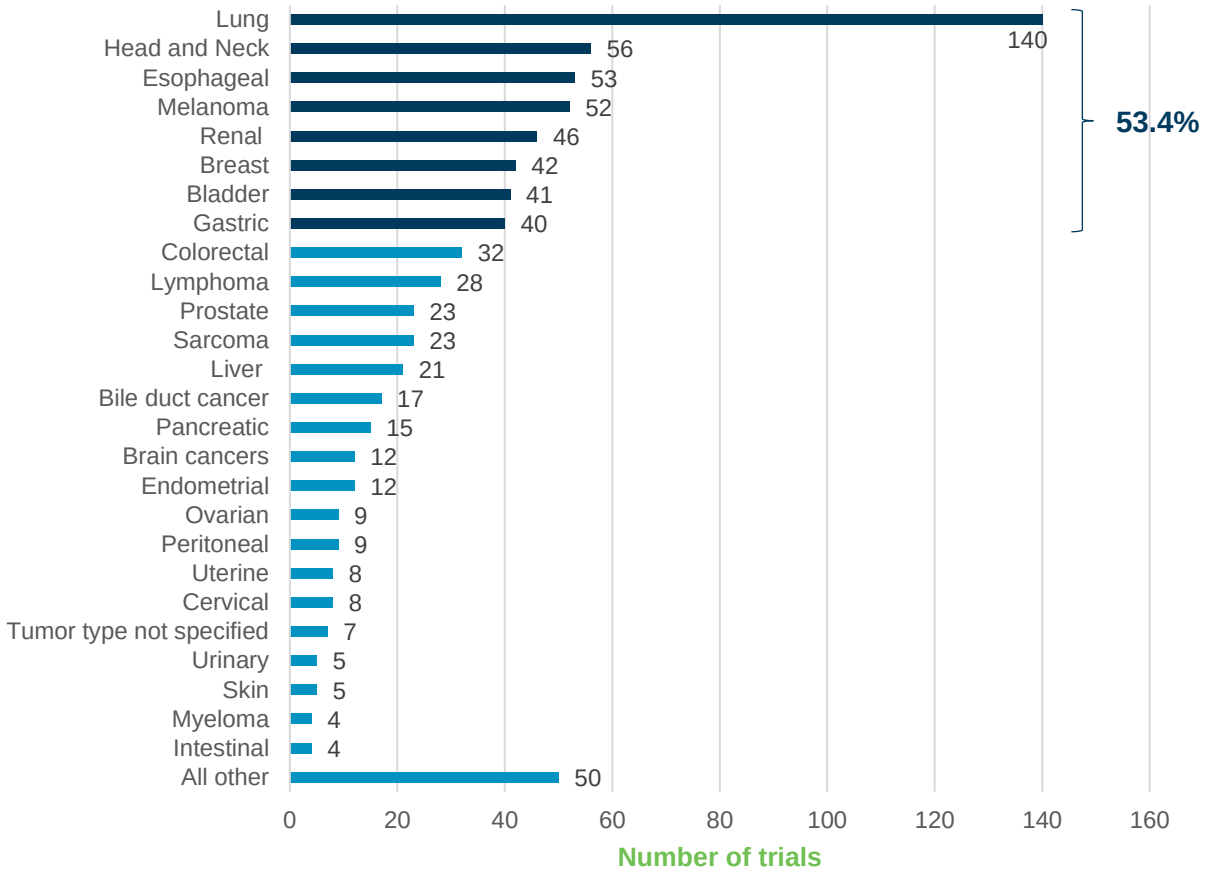


Figure 4 Overview of tumor indications assessed in ICI combination trials between Jan 1st, 2019 and Jun 30th, 2020. *Please note that some trials assessed >1 tumor indication/trial.

Clinical data and regulatory approvals of ICI combination therapies

The most commonly recognized and studied inhibitory checkpoint pathways thus far are the PD-1/programmed death ligand-1(PD-L) and cytotoxic T lymphocyte-associated molecule-4 (CTLA-4) pathways (Chen and Melman, 2013; Marin-Acevedo et al, 2018; Schmidt, 2019). While promising initial results were obtained with ICI monotherapy, leading to approval of several antibodies targeting the PD-1/PD-L1 and CTLA-4 pathways as early as 2011

(**Appendix A; Table 1**), the clinical utility of ICIs is being expanded by investigating checkpoint inhibitors in combination.

Conventional cancer treatment modalities, such as radio- or chemotherapy, or more novel targeted therapies achieve antitumoral effects via mechanisms and pathways different from those employed by ICIs (**Figure 1B**). With this in mind, ICI combination therapies are extensively evaluated in clinical trials for their potential synergistic or additive effects, ultimately leading to supplemental approvals (by the FDA) or extensions of indications for most of the initially approved ICIs (by the European Medicines Agency [EMA]) (**Appendix A; Table 1**).

ICIs and targeted therapies

ICIs plus targeted therapies were (Draghiciu et al, 2019) and continue to be (**Figure 3A**) investigated in a large variety of tumor types due to the potential to target several oncogenic pathways, including for example angiogenesis and DNA repair response that will be highlighted in the following paragraphs.

One of the main factors involved in angiogenesis is the vascular endothelial growth factor (VEGF), which stimulates tissue remodeling and is reported to have immunosuppressive effects that render it a candidate target to potentiate ICI antitumoral immune responses (Mansfield et al, 2013; Ott et al, 2015). From the plethora of clinical trials assessing ICI and VEGF-targeted therapies thus far, the pivotal Keynote 426, Impower150, and JAVELIN 101 Phase III trials are of particular relevance. In the Keynote 426 trial, an ICI combination therapy consisting of the anti-PD-1 antibody pembrolizumab plus axitinib, which targets the VEGF receptor, versus standard of care was evaluated in 861 patients with previously untreated advanced renal cell carcinoma (RCC). The combined therapy led to an almost 24% difference in ORR and an almost 12% difference in PFS across all risk groups and regardless of PD-L1 expression (Rini et al, 2019). These results highlight a substantial improvement in both response rates and durability of responses, thus leading to approval of the combination for treatment of patients with advanced RCC (**Appendix A; Table 1**). The Impower150 trial assessed the anti-PD-L1 ICI atezolizumab in combination with the VEGF-targeting therapy bevacizumab and chemotherapy in 1,045 NSCLC patients without endothelial growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) tumor mutations. Results reported a median overall survival (OS) of 19.2 versus 14.7 months and a median PFS of 8.5 versus 7.0 months in the ICI combined therapy versus control (bevacizumab plus chemotherapy) groups, with an increase in the median duration of response (DOR) from 6.5 to 10.8 months (Socinski et al, 2018). Based on these results, and considering the acceptable safety profile, this ICI combination therapy was approved by both the FDA and the EMA for first-line treatment of metastatic non-squamous NSCLC (**Appendix A; Table 1**). Similarly, the results of the JAVELIN 101 trial assessing the anti-PD-L1 ICI avelumab in combination with axitinib led to its approval for first-line treatment of patients with advanced RCC (**Appendix A; Table 1**). Trial results reported a statistically significant improvement in PFS in the total population, with a median PFS of 13.8 versus 8.4 months in the ICI combination therapy versus single-treatment control arms (Motzer et al, 2019).

The second pathway to highlight is directly related to increasing the tumor mutational burden and tumor-specific antigen release. The DNA damage repair machinery plays a crucial role in cell cycle regulation and tumorigenesis (Jackson et al, 2009), and with its inhibition potentially increasing the TMB of tumors by preventing DNA damage repair (Yan et al, 2018). In that context, several clinical trials evaluating poly (ADP-ribose) polymerase (PARP) inhibitors in combination with ICIs have been initiated in the last five years in various solid tumors, including NSCLC and small cell lung cancer (SCLC), breast cancer, and cancers of the gastrointestinal tract, many of which are currently still ongoing (see Yan et al, 2018). Since the recent approval

for the treatment of ovarian cancers harboring BRCA mutations, the PARP inhibitor olaparib has been investigated in combination with ICIs for the treatment of patients with other solid tumors. The results of an ongoing Phase II trial evaluating the combined treatment of olaparib and anti-PD-L1 monoclonal antibody durvalumab were recently published (Thomas et al, 2019). Of the evaluable patients with SCLC, around 21% had clinical benefit following combined treatment, with around 11% presenting with partial or complete responses. These are promising results for patients with SCLC, who continue to have one of the worst survival rates (<6%) of all patients with cancer (Govindan et al, 2006; Imai et al, 2016). Interestingly, all patients responding to treatment presented with an "inflamed" immune phenotype characterized by the presence of CD8+ T cells in the tumors, intratumoral PD-L1 expression, and high mutational tumor load (Thomas et al, 2019). These results may also suggest that high intratumoral PD-L1 expression together with the presence of antitumor immune cells and a high TMB, could be used as markers predictive of response to treatment. Other clinical trials investigating ICI combination therapies with olaparib are currently ongoing in ovarian cancer (NCT04015739) and bladder cancer (Phase II NEODURVARIB trial, NCT03534492; Rodriguez-Moreno et al, 2020).

Combination therapies of multiple ICIs

A different investigated strategy for simultaneous blockade of several oncogenic pathways assesses the combination of two or more ICIs targeting different checkpoint molecules and pathways (see **Figure 1B**) (Schmidt, 2019). This combinatorial approach was initially evaluated in patients with melanoma, where treatment with the anti-PD-1 monoclonal antibody nivolumab in combination with the anti-CTLA-4 antibody ipilimumab in a pivotal Phase III trial led to a PFS of 11.5 versus 2.9 months following combination therapy versus ipilimumab alone (CheckMate 067, Larkin et al, 2015). In PD-L1-positive patients, the median PFS was 14.0 months in both treatment groups, but in the PD-L1-negative patient subgroup, PFS was longer with the combination approach compared with nivolumab alone (11.2 versus 5.3 months). These promising results led to the first approval of nivolumab and ipilimumab as combined therapy (**Appendix A; Table 1**) and fueled subsequent research in other solid tumors. The Phase III CheckMate 214 trial, performed in intermediate and poor-risk patients with previously untreated advanced RCC, demonstrated the clinical superiority of nivolumab plus ipilimumab over sunitinib (18-month ORR: 75% versus 60%, median OS: not reached versus 26.0 months; Motzer et al, 2018), thus leading to the approval of this combination in this patient population (**Appendix A; Table 1**). Similar pivotal trials were performed in patients with metastatic colorectal cancer (CRC; Phase II CheckMate 142 trial: ORR of 55% following combined therapy versus 28% following nivolumab alone; Morse et al, 2019) and hepatocellular carcinoma (HCC; Phase I/II CheckMate 040 trial: ORR of 31-32%, depending on the concentrations of the compounds used in the combined therapy; Yau et al, 2019). The remarkable results recorded in these trials led to FDA-accelerated approvals for use of nivolumab plus ipilimumab in patients with metastatic CRC in 2018 and in patients with HCC in 2020 (**Appendix A; Table 1**).

ICIs and standard of care therapy

The second and third most frequently investigated combination therapies between 2019 and 2020 that include ICIs consist of standard of care treatments, chemotherapy and radiotherapy, which have been the backbone of anti-cancer therapy in the clinic for decades.

Cytotoxic chemotherapy has been speculated to have immunostimulatory properties that may enhance the antitumoral effects of ICIs (Coffelt et al, 2015) by increasing tumor immunogenicity, disrupting immune suppressive pathways, inducing immunogenic cell death, and potentiating T cell responses (Zitvogel et al, 2013; Galluzzi et al, 2015; Apetoh et al, 2015;

Gotwals et al, 2017). Combination therapies of ICIs and chemotherapy have been studied primarily in NSCLC, providing expanded treatment options with improved outcomes. The 2017 FDA approval of pembrolizumab combined with carboplatin/(nab)paclitaxel for treatment of patients with squamous NSCLC (**Appendix A; Table 1**) has encouraged the investigation of ICI and chemotherapy combinations in other types of cancers within the last years. The results obtained from the Phase III Keynote 048 trial in patients with metastatic/unresectable recurrent head and neck squamous cell carcinoma, namely a median OS of 13.0 vs 10.7 months following combined pembrolizumab and platinum plus 5-fluorouracil (5-FU) versus cetuximab combined with the same chemotherapy (Burtness et al, 2019), demonstrated the superiority of using the ICI chemotherapy combination and led to the approval as front-line treatment for this patient population in 2019 (**Appendix A; Table 1**). The most recent approval of a combination therapy of ICI plus chemotherapy was granted by the FDA in March 2020 for durvalumab in association with etoposide and cisplatin/carboplatin for first-line treatment of patients with extensive-stage SCLC (**Appendix A; Table 1**). The approval was based on the results of the Phase III CASPIAN trial, which reported a median OS of 13.0 versus 10.3 months in the combined therapy versus chemotherapy alone groups (Paz-Ares et al, 2019). Together with the increased amount of clinical trials initiated since 2019 that focus on ICI plus chemotherapy treatment regimens (**Figure 3A**), this highlights their central roles in cancer treatment.

Similar to chemotherapy, radiotherapy remains a backbone treatment modality for different types of cancer. Its main mechanisms of action are induction of single- and double-stranded DNA breaks and activation of multiple signaling pathways (Formenti et al, 2009), ultimately leading to tumor cell death that can further initiate systemic antitumoral immune responses and trigger abscopal effects (Siva et al, 2013). The putative synergy in antitumoral activity of radiotherapy and ICIs (Sharabi et al, 2015) has led to enhanced clinical research efforts in the past years (Draghiciu et al, 2019; **Figure 3A**). For example, ipilimumab in combination with radiotherapy evaluated in a single-arm Phase II trial in patients with melanoma and unresectable brain metastases led to a 1-year OS of 31.8%, which is higher than historically reported results for this patient population (Lopez-Martin et al, 2018). Nevertheless, clinical data supporting the routine application of ICIs combined with only radiotherapy remains limited and approaches including both chemo- and radiotherapy in association with ICIs have proven a better efficacy (Antonia et al, 2017). For example, the Phase III randomized PACIFIC trial investigating the role of durvalumab therapy following chemoradiation of 713 stage III NSCLC patients reported an 18-month PFS rate of 44.2% versus 27.0% in patients receiving durvalumab versus placebo (Antonia et al, 2017), thus leading to approval of this compound in NSCLC (**Appendix A; Table 1**). Various radiotherapy and ICI combination therapies are currently being evaluated in other tumor types in clinical trials initiated since 2019, such as nasopharyngeal (NCT03907826) and hepatocellular (NCT04167293) carcinomas or endometrial (NCT04214067) and colorectal (NCT03927898) cancers.

Clinical development of next-generation checkpoint modulators

While first-generation ICIs have revolutionized cancer treatment, targeting of novel pathways with the help of next-generation ICIs may further benefit patients. In recent years, the focus shifted towards direct lymphoid targeting agents and agents targeting the tumor microenvironment (TME) or antigen-presenting cell (APC) maturation processes. These two classes of agents as next-generation ICIs are being investigated as mono- as well as combination-therapies.

The most investigated next-generation lymphoid checkpoint modulators for combination therapies are lymphocyte-activation gene 3 (LAG-3), T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), and CD73, which are often co-

expressed with PD-1/CTLA-4 but play different roles in specific anatomic locations (Anderson et al, 2016).

Our analysis shows that, currently, indoleamine-2,3-doxygenase 1 (IDO1) and colony stimulating factor 1 receptor (CSF-1R) are two TME modulating agents that are actively being investigated in combination trials with ICIs.

LAG-3 downmodulates lymphocyte responses in infection and cancer (Woo et al, 2012). Of the four anti-LAG-3 antibodies currently in clinical development, relatlimab is the most advanced and has also been assessed in combination therapies. In pretreated melanoma patients, relatlimab plus nivolumab therapy led to an 11.5% ORR and 49% disease control rate, with responses correlating with LAG-3 expression levels, irrespective of PD-L1 expression (Ascierto et al, 2017). Furthermore, the toxicity of combined therapy was similar to that of nivolumab alone or historical controls (Ascierto et al, 2017), thus highlighting the remarkable results for a patient population with no therapeutic options up to now. These promising results fueled recent investigations of relatlimab plus nivolumab in other solid tumors (**Appendix A; Table 2**), with a total of 11 clinical trials starting in 2019.

TIGIT interferes with the co-stimulatory axis composed of the CD226 receptor expressed on NK cells, T cells, and monocytes and CD155 and CD122 as ligands, expressed on dendritic cells and tumor cells (Anderson et al, 2017). In patients with melanoma, TIGIT was shown to synergize with PD-1 (Chauvin et al, 2015; Kurtulus et al, 2015), with TIGIT-PD-1 co-blockade leading to increased CD8+ T cell proliferation and cytokine production (Chauvin et al, 2015). More recently, TIGIT expression in advanced cancers such as breast, colon, and lung, was reported to be associated with PD-L1 expression (Pal et al, 2018). From the anti-TIGIT combination therapies assessed thus far, tiragolumab plus atezolizumab therapy has progressed to Phase III clinical trials in NSCLC or SCLC. Both Phase III trials were initiated in 2020 (**Appendix A; Table 2**), with results expected in 2023-2025.

Signaling mediators of the TIM-3 glycoprotein remain to be completely elucidated. TIM-3 is upregulated in tumor-infiltrating lymphocytes in lung, gastric, head and neck cancers, melanoma, and B-cell non-Hodgkin lymphoma, and has multiple biologically heterogenic ligands, such as carbohydrate-binding galectin-9 with immunosuppressive functions (Chiba et al, 2012; Huang et al, 2015) and the carcinoembryonic antigen-related cell adhesion molecule 1 (Huang et al, 2015). Similar to LAG-3, TIM-3 is usually co-expressed with PD-1 by exhausted T cells and its blockade synergizes with anti-PD-1 (Han et al, 2013). Of the anti-TIM-3 monoclonal antibodies investigated in clinical trials, four are currently evaluated in combination therapies in solid tumors, lymphoma or myelodysplastic syndromes (MDS; **Appendix A; Table 2**). Starting 2019, three Phase II trials have been initiated, assessing TSR-022 combination therapies in hepatocellular carcinoma and melanoma and assessing MBG453 combination therapies in MDS. The results reported thus far are from the Phase Ib trial evaluating MBG453 in combination with decitabine in patients with high-risk MDS (NCT03066648), where 50% of patients achieved complete responses, with none of the responding patients having disease recurrence (Borate et al, 2019). In June 2020, the first Phase III trial with MBG453 in combination with azacytidine was initiated in patients with high-risk MDS (**Appendix A; Table 2**).

Another lymphoid target in development is CD73, an enzyme expressed on the surface of cancer cells and whose main role is to convert AMP to free adenosine, which in turn contributes to the inhibition of cellular immune responses and tumor immune escape (Kordas et al, 2018). While CD73 is overexpressed both in multiple solid tumors and leukemias, and its inhibition enhances the activity of cytotoxic lymphocytes, combination with other therapies is required for achieving significant survival advantage (Young et al, 2016). The initially reported synergy with anti-PD-1/PD-L1 or anti-CTLA-4 antibodies in preclinical studies (Iannone et al, 2014; Hay et al, 2016; Willingham et al, 2018) led to evaluation of such combination therapies in early-phase clinical trials. Preliminary results from a first-in-human Phase I trial with the anti-CD73 antibody oleclumab combined with durvalumab in patients with advanced CRC reported encouraging activity of the combination, with sustained reduction in soluble and T-cell expressed CD73 across all doses and patients (Overman et al, 2018). This, together with the

manageable safety profile, led to the continued clinical evaluation of this combination (**Appendix A; Table 2**), in three clinical trials initiated since 2019.

Several types of non-lymphoid cells present in the tumor microenvironment have been reported to play crucial roles in the activation, intratumoral infiltration, and cytotoxic activity of antitumor immune cells (Mantovani et al, 2017; Belli et al, 2018). In this context, using next-generation checkpoint modulators to target key enzymes or growth factor receptors expressed by non-lymphoid cells that negatively regulate antitumor immune responses is a potential avenue for improving treatment outcome.

IDO1 is a cytosolic enzyme that inhibits immune activation through several mechanisms: direct signaling through its ITIM domain (Mbongue et al, 2015), inhibition of the mammalian target of rapamycin (mTOR) by depleting tryptophan (Hwu et al, 2000; Kudo et al, 2001; Frumento et al, 2001), and stimulating kynurenine formation, which potentiates regulatory T cell (Treg) activity (Gutierrez-Vazquez et al, 2017). Furthermore, IDO1 was shown to mediate both primary and acquired resistance to anti-CTLA4 therapy (Spranger et al, 2013; Holmgaard et al, 2013), specifically with tumors expressing this enzyme developing potent immune resistance mechanisms (Uyttenhove et al, 2003). Of the multitude of agents currently in clinical investigations for targeting IDO1, next-generation checkpoint agents structurally related to tryptophan, such as indoximod (D-methyl-tryptophan), have proven the greatest success in combination therapies thus far. In a Phase II randomized trial assessing indoximod in combination with the dendritic cell vaccine sipuleucel-T in patients with metastatic resistant prostate cancer (**Appendix A; Table 2**), an increase in PFS from 4.1 to 10.3 months was reported in the combined indoximod plus sipuleucel-T versus the single sipuleucel-T treatment arm (Jha et al, 2017). Furthermore, in patients with pancreatic adenocarcinoma (**Appendix A; Table 2**), the combination of indoximod and gemcitabine and nab-paclitaxel showed preliminary efficacy in a Phase II trial, with an ORR of 37% (Bahary et al, 2016). In view of these promising preliminary results, indoximod is currently being evaluated in combination therapies with chemoradiotherapy (**Appendix A; Table 2**). Another IDO1 inhibitor is epacadostat, an IDO1-selective hydrozyamidine reported to have low toxicity at pharmacologically effective doses in a Phase I clinical trial (Beatty et al, 2017). The further clinical evaluations of epacadostat in combination with other therapies in larger clinical trials was initiated, of which the ECHO-301 Phase III trial is of particular relevance. Assessment of epacadostat combined with pembrolizumab in patients with melanoma in the ECHO-301 trial failed to show superiority over pembrolizumab alone (Long et al, 2018). These results triggered a slowdown in the clinical development of all IDO1 inhibitors during the course of 2018 and beyond, with only two trials initiated with epacadostat combination therapy starting in 2019 (**Appendix A; Table 2**). A promising IDO1 inhibitor is BMS-986205, a fluoroquinolone derivative with higher potency and stability as compared with other IDO1 inhibitors (Prendergast et al, 2017). In a Phase I/II trial in patients with solid tumors, BMS-986205 in combination with nivolumab led to an ORR of 50% in patients with tumor PD-L1 expression ≥1% versus 30% in patients with tumor PD-L1 expression <1% (Luke et al, 2017). This highlighted the therapeutic benefit of combining BMS-986205 with nivolumab, a therapy currently investigated in endometrial cancer (**Appendix A; Table 2**).

Another target in development with immune checkpoint inhibitors is CSF-1R. CSF-1R is a tyrosine kinase receptor belonging to the platelet-derived growth factor family, and whose inhibition was shown to modulate both the phenotype and recruitment of tumor-associated macrophages (TAMs), thus potentiating the efficacy of PD-1/PD-L1, CTLA-4, or IDO1 inhibition (Ries et al, 2014; Cannarile et al, 2017). This is of particular relevance given the capacity of T cells to secrete CSF-1 upon PD-1 inhibition, a reported mechanism of acquired resistance (Eissler et al, 2016). Pharmacological inhibition of CSF-1R is currently under investigation, with a particular focus on ovarian cancer, pancreatic cancer or glioma, in which TAMs play a crucial role (Zhu et al, 2014; Quail et al, 2016). The main CSF-1R targeting agent investigated thus far in combination therapies with promising preliminary results is emactuzumab. In a recent Phase I clinical trial in patients with solid tumors including ovarian cancer (**Appendix A; Table 2**), combined emactuzumab and paclitaxel-based chemotherapy revealed depletion of

immunosuppressive M2-like TAMs (Gomez-Roca et al, 2019). These results fueled further evaluations of emactuzumab and paclitaxel in ovarian cancer, alone or in association with bevacizumab, in a currently ongoing Phase II trial (**Appendix A; Table 2**), with results expected in the near future.

Conclusions

The results of our analysis and overview offer a broad and detailed summary of the recent trends in ICI combination trials, thus representing a tool for obtaining an exhaustive view of the present status and new advances in the immuno-oncology checkpoint inhibitor and modulator field. The in-depth understanding of the recent trial landscape of ICIs in combination therapies, is crucial in guiding the development and design of future clinical trials for novel ICI combinations in tailored approaches for specific disease types and subsets of selected patients for an optimized cancer management. The results of our analysis support the selection and implementation of the most suitable changes in trial design, objectives and proof-of-concept read-outs. Lastly, the anticipated results of ongoing Phase II/III trials with next-generation ICIs will confirm their potential as novel therapeutic modalities for oncology patients, contributing to overall anticipated changes in the current cancer treatment paradigm.

Immunotherapy is a mainstay in cancer therapy and will remain a fast evolving and complex field. This is despite the impact of the COVID-19 pandemic (Upadhaya S et al, 2020). Navigating the continuous development will depend on clear medical and biological rationales to direct the clinical strategies supporting their translation ‘from bench to bedside’.

References

Anderson AC, Joller N, Kuchroo VK, Hospital W, Immunology E. Lag-3, Tim-3, and TIGIT: Co-inhibitory receptors with specialized functions in immune regulation 2017;44: 989e1004.

Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, Yokoi T, Chiappori A, Lee KH, de Wit M, Cho BC, Bourhaba M, Quantin X, Tokito T, Mekhail T, Planchard D, Kim YC, Karapetis CS, Hiret S, Ostoros G, Kubota K, Gray JE, Paz-Ares L, de Castro Carpeño J, Wadsworth C, Melillo G, Jiang H, Huang Y, Dennis PA, Özgüroğlu M, PACIFIC Investigators. Durvalumab after Chemoradiotherapy in Stage III Non-Small-Cell Lung Cancer. N Engl J Med. 2017 Nov 16; 377(20):1919-1929.

Apetoh L, Ladoire S, Coukos G, Ghiringhelli F. Combining immunotherapy and anticancer agents: the right path to achieve cancer cure? Ann Oncol (2015) 26(9):1813–23.10.1093/annonc/mdv209.

Ascierto PA, Bono P, Bhatia S, Melero I, Nyakas MS, Svane IM, et al. LBA18Efficacy of BMS-986016, a monoclonal antibody that targets lymphocyte activation gene-3 (LAG-3), in combination with nivolumab in pts with melanoma who progressed during prior antiePD-1/PD-L1 therapy (mel prior IO) in all-comer and biomarker-enriched. Ann Oncol 2017;28:mdx440emdx011.

Bahary N, Garrido-Laguna I, Cinar P, O’Rourke MA, Somer BG, Nyak-Kapoor A, et al. Phase 2 trial of the indoleamine 2,3-dioxygenase pathway (Ido) inhibitor indoximod plus gemcitabine/nab-paclitaxel for the treatment of metastatic pancreas cancer: interim analysis. J Clin Oncol 2016;34:3020.

Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. Lancet. 2017;389(10064):67–76.

Beatty GL, O’Dwyer PJ, Clark J, Shi JG, Bowman KJ, Scherle PA, et al. First-in-Human phase I study of the oral inhibitor of indoleamine 2,3-dioxygenase-1 Epacadostat (INCB024360) in patients with advanced solid malignancies. Clin Cancer Res 2017;23:3269e76.

Belli C, Trapani D, Viale G, D’Amico P, Duso BA, Della Vigna P, et al. Targeting the microenvironment in solid tumors. Cancer Treat Rev 2018;65:22e32.

Borate U, Esteve J, Porkka K, et al. Phase Ib Study of the Anti-TIM-3 Antibody MBG453 in Combination with Decitabine in Patients with High-Risk Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukemia (AML). Blood (2019) 134 (Supplement_1): 570.

Burtness B, Harrington KJ, Greil S, et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. Lancet. 2019 Nov 23;394(10212):1915-1928.

Campesato LF, Barroso-sousa R, Jimenez L, Camargo AA. Comprehensive cancer-gene panels can be used to estimate mutational load and predict clinical benefit to PD-1 blockade in clinical practice. Oncotarget. 2015;6(33):34221.

Cannarile MA, Weisser M, Jacob W, Jegg A-M, Ries CH, Ru¨ ttinger D. Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. J Immunother Cancer 2017;5:53.

Carbone David P., Reck Martin, Paz-Ares Luis, Creelan Benjamin, Horn Leora, Steins Martin, Felip Enriqueta, van den Heuvel Michel M., Ciuleanu Tudor-Eliade, Badin Firas, Ready Neal, Hiltermann T. Jeroen N., Nair Suresh, Juergens Rosalyn, Peters Solange, Minenza Elisa, Wrangle John M., Rodriguez-Abreu Delvys, Borghaei Hossein, Blumenschein George R., Villaruz Liza C., Havel Libor, Krejci Jana, Corral Jaime Jesus, Chang Han, Geese William J., Bhagavatheeswaran Prabhu, Chen Allen C., Socinski Mark A. First-Line Nivolumab in Stage IV or Recurrent Non–Small-Cell Lung Cancer. New England Journal of Medicine. 2017;376(25):2415–2426.

Chauvin J-M, Pagliano O, Fourcade J, Sun Z, Wang H, Sander C, et al. TIGIT and PD-1 impair tumor antigen-specific CD8+ T cells in melanoma patients. J Clin Invest 2015;125:2046e58.

Chiba S, Baghdadi M, Akiba H, Yoshiyama H, Kinoshita I, Dosaka-Akita H, et al. Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1. Nat Immunol 2012;13:832e42.

Coffelt SB, de Visser KE. Immune-mediated mechanisms influencing the efficacy of anticancer therapies. Trends Immunol (2015) 36(4):198–216.10.1016/j.it.2015.02.006

Cogdill AP, Andrews MC, Wargo JA. Hallmarks of response to immune checkpoint blockade. Br. J. Cancer. 117: 1–7 (2017).

Draghiciu O, Feenstra R, Pawlitzky I. Combination approaches in immune-oncology trials: new horizons for multiple tumor indications. J Clin Studies. V11 I4 (2019).

Dunn GP, Sheehan KC, Old LJ, Schreiber RD. IFN unresponsiveness in LNCaP cells due to the lack of JAK1 gene expression. Cancer Res. 2005;65:3447-53.

Eissler N, Mao Y, Brodin D, Reuterswa¨rd P, Andersson Svahn H, Johnsen JI, et al. Regulation of myeloid cells by activated T cells determines the efficacy of PD-1 blockade. OncoImmunology 2016;5:e1232222.

Eroglu Z, Zaretsky JM, Hu-Lieskovan S, Kim DW, Algazi A, Johnson DB, et al. High response rate to PD-1 blockade in desmoplastic melanomas. Nature. 2018;553(7688):347–350.

Formenti SC, Demaria S. Systemic effects of local radiotherapy. Lancet Oncol. 2009 Jul; 10(7):718-26.

Francello L, Gandini S, Pelicci PG, Mazarella L. Tumor mutational burden quantification from targeted gene panels: major advancements and challenges. J Immunother Cancer. 7:183 (2019).

Frumento G, Rotondo R, Tonetti M, Ferrara GB. T cell proliferation is blocked by indoleamine 2,3-dioxygenase. Transplant Proc 2001;33:428e30.

Gomez-Roca CA, Italiano A, Le Tourneu C, et al. Phase I study of emactuzumab single agent or in combination with paclitaxel in patients with advanced/metastatic solid tumors reveals depletion of immunosuppressive M2-like macrophages. Ann Oncol. 2019 Aug 1;30(8):1381-1392.

Gotwals P, Cameron S, Cipolletta D, Cremasco V, Crystal A, Hewes B, et al. Prospects for combining targeted and conventional cancer therapy with immunotherapy. Nat Rev Cancer (2017) 17(5):286–301.10.1038/nrc.2017.17.

Govindan R, Page N, Morgensztern D, et al. Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. J Clin Oncol 2006;24:4539–44.

Gutierrez-Vazquez C, Quintana FJ. Regulation of the immune response by the Aryl Hydrocarbon receptor. Immunity 2018;48: 19e33.

Han G, Chen G, Shen B, Li Y. Tim-3: an activation marker and activation limiter of innate immune cells. Front Immunol 2013;4: 449.

Hay CM, Sult E, Huang Q, Mulgrew K, Fuhrmann SR, McGlinchey KA, et al. Targeting CD73 in the tumor microenvironment with MEDI9447. OncoImmunology 2016;5:1e10. https://doi.org/10.1080/2162402X.2016.1208875.

Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363:711-23.

Holmgaard RB, Zamarin D, Munn DH, Wolchok JD, Allison JP. Indoleamine 2,3-dioxygenase is a critical resistance mechanism in antitumor T cell immunotherapy targeting CTLA-4. J Exp Med 2013;210. 1389 LP e 1402.

Huang Y-H, Zhu C, Kondo Y, Anderson AC, Gandhi A, Russell A, et al. CEACAM1 regulates TIM-3-mediated tolerance and exhaustion. Nature 2015;517:386e90.

Hugo W, Zaretsky JM, Sun L, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. Cell. 2016;165:35-44.The DNA-damage response in human biology and disease. Jackson SP, Bartek J. Nature. 2009 Oct 22; 461(7267):1071-8.

Hwu P, Du MX, Lapointe R, Do M, Taylor MW, Young HA. Indoleamine 2,3-dioxygenase production by human dendritic cells results in the inhibition of T cell proliferation. J Immunol 2000;164:3596e9.

Iannone R, Miele L, Maiolino P, Pinto A, Morello S. Adenosine limits the therapeutic effectiveness of anti-CTLA4 mAb in a mouse melanoma model. Am J Cancer Res 2014;4:172e81.

Imai H, Mori K, Watase N, et al. Clinical significance of the relationship between progression-free survival or postprogression survival and overall survival in patients with extensive disease-small-cell lung cancer treated with carboplatin plus etoposide. Can Respir J 2016;2016:5405810.

Jha GG, Gupta S, Tagawa ST, Koopmeiners JS, Vivek S, Dudek AZ, et al. A phase II randomized, double-blind study of sipuleucel-T followed by Ido pathway inhibitor, indoximod, or placebo in the treatment of patients with metastatic castration resistant prostate cancer (mCRPC). J Clin Oncol 2017;35:3066.

Johnson D. B., Frampton G. M., Rioth M. J., Yusko E., Xu Y., Guo X., Ennis R. C., Fabrizio D., Chalmers Z. R., Greenbowe J., Ali S. M., Balasubramanian S., Sun J. X., He Y., Frederick D. T., Puzanov I., Balko J. M., Cates J. M., Ross J. S., Sanders C., Robins H., Shyr Y., Miller V. A., Stephens P. J., Sullivan R. J., Sosman J. A., Lovly C. M. Targeted Next Generation Sequencing Identifies Markers of Response to PD-1 Blockade. Cancer Immunology Research. 2016;4(11):959–967.

Kordas T, Osen W, Eichmüller SB (2018-04-18). "Controlling the Immune Suppressor: Transcription Factors and MicroRNAs Regulating CD73/NT5E". Frontiers in Immunology. 9: 813.

Koyama S, Akbay EA, Li YY, et al. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun. 2-16;7:10501.

Kudo Y, Boyd CA, Sargent IL, Redman CW. Tryptophan degradation by human placental indoleamine 2,3-dioxygenase regulates lymphocyte proliferation. J Physiol 2001;535:207e15.

Kurtulus S, Sakuishi K, Ngiow S-F, Joller N, Tan DJ, Teng MWL, et al. TIGIT predominantly regulates the immune response via regulatory T cells. J Clin Invest 2015;125:4053e62.

Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Previously Untreated Melanoma. N Engl J Med. 2015 Jul 2; 373(1): 23–34.

Le DT, Uram JN, H W, R BB, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. NEJM. 2015:2509–20.

Long GV, Dummer R, Hamid O, et al. Epacadostat (E) plus pembrolizumab (P) versus pembrolizumab alone in patients (pts) with unresectable or metastatic melanoma: Results of the phase 3 ECHO-301/KEYNOTE-252 study. Journal of Clin Oncol, volume 36, Issue 15 (suppl), abstract 108.

Lopez-Martin JA, Arance AM, De La Cruz-Merino L, Illescas A, Valduvieco I, Berrocal A, et al. Ipilimumab and radiation in patients with unresectable melanoma brain metastases: a multicenter, open label, phase-2, Spanish melanoma group (GEM) study (NCT-2013-001132-22). J Clin Oncol (2018) 36(Suppl; abstr 9546).

Luke JJ, Gelmon K, Pachynski RK, Desai J, Moreno V, Tabernero JM, et al. O41 Preliminary antitumor and immunomodulatory activity of BMS-986205, an optimized indoleamine 2,3-dioxygenase 1 (Ido1) inhibitor, in combination with nivolumab in patients with advanced cancers. J Immunother Cancer 2017;5:89.

Mansfield AS, Nevala WK, Lieser EA, Leontovich AA, Markovic SN. The immunomodulatory effects of bevacizumab on systemic immunity in patients with metastatic melanoma. Oncoimmunology. 2013 May 1; 2(5):e24436.

Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. Nat Rev Clin Oncol 2017;14:399e416.

Marin-Acevedo JA, Dholaria B, Soyano AE, et al. Next generation of immune checkpoint therapy in cancer: new developments and challenges. J Hematol Oncol. 2018; 11: 39.

Mbongue JC, Nicholas DA, Torrez TW, Kim N-S, Firek AF, Langridge WHR. The role of indoleamine 2, 3-dioxygenase in immune suppression and autoimmunity. Vaccines 2015;3: 703e29.

Morse M, Overman MJ, Hartman L, et al. Safety of Nivolumab plus Low-Dose Ipilimumab in Previously Treated Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer. Oncologist. 2019 Nov;24(11):1453-1461.

Motzer RJ, Tannier NM, McDermott DF, et al. Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. N Engl J Med. 2018 Apr 5;378(14):1277-1290.

Motzer RJ, Penkov K, Haanen J, et al. Avelumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. N Engl J Med 2019; 380:1103-1115.

Ott PA, Hodi FS, Buchbinder EI. Inhibition of Immune Checkpoints and Vascular Endothelial Growth Factor as Combination Therapy for Metastatic Melanoma: An Overview of Rationale, Preclinical Evidence, and Initial Clinical Data. Front Oncol. 2015; 5():202.

Overman MJ, Lorusso P, Strickler JH, Patel SP, Clarke SJ, Noonan AM, et al. Safety, efficacy and pharmacodynamics (PD) of MEDI9447 (oleclumab) alone or in combination with durvalumab in advanced colorectal cancer (CRC) or pancreatic cancer (panc). J Clin Oncol 2018;36(suppl). abstr 4123.

Pal SK, Vanderwalde AM, Szeto C, et al. PD-L1 expression is strongly associated with TIGIT, FoxP3 and Lag3 across advanced cancers, but not OX40, TIM-3, and IDO. Annals of Oncology (2018) 29 (suppl_8): viii400-viii441. 10.1093/annonc/mdy288.

Paz-Ares L, Dvorkin M, Chen Y, et al. Durvalumab plus platinum–etoposide versus platinum–etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial. ARTICLES| VOLUME 394, ISSUE 10212, P1929-1939, NOVEMBER 23, 2019.

Powles Thomas, Durán Ignacio, van der Heijden Michiel S, Loriot Yohann, Vogelzang Nicholas J, De Giorgi Ugo, Oudard Stéphane, Retz Margitta M, Castellano Daniel, Bamias Aristotelis, Fléchon Aude, Gravis Gwenaëlle, Hussain Syed, Takano Toshimi, Leng Ning, Kadel Edward E, Banchereau Romain, Hegde Priti S, Mariathasan Sanjeev, Cui Na, Shen Xiaodong, Derleth Christina L, Green Marjorie C, Ravaud Alain. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. The Lancet. 2018;391(10122):748–757.

Prendergast GC, Malachowski WP, DuHadaway JB, et al. Discovery of IDO1 inhibitors: from bench to bedside. Cancer Res. 2017 December 15; 77(24): 6795–6811.

Quail DF, Bowman RL, Akkari L, Quick ML, Schuhmacher AJ, Huse JT, et al. The tumor microenvironment underlies acquired resistance to CSF-1R inhibition in gliomas. Science (80-) 2016:352.

Restifo NP, Smyth MJ, Snyder A. Acquired resistance to immunotherapy and future challenges. Nat Rev Cancer. 2016;16:121-6.

Ries CH, Cannarile MA, Hoves S, Benz J, Wartha K, Runza V, et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. Cancer Cell 2014;25:846e59.

Rini B, Plimack E, Stus V, et al. Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. N Engl J Med 2019; 380:1116-1127.

Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non – small cell lung cancer. Science. 2015;348(6230):124–129.

Robert C, Schachter J, Long GV, et al; KEYNOTE-006 investigators. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med. 2015;372:2521-32.

Rodriguez-Moreno JF, de Velasco G, Bravo Fernandez I, et al. Impact of the combination of durvalumab (MEDI4736) plus olaparib (AZD2281) administered prior to surgery in the molecular profile of resectable urothelial bladder cancer: NEODURVARIB Trial. Presented at: GU Cancers Symposium; February 13-15, 2020; San Francisco, CA. Abstract 542.

Schmidt EV. Developing combination strategies using PD-1 checkpoint inhibitors to treat cancer. Semin Immunopathol. 2019; 41(1): 21–30.

Sharabi AB, Lim M, DeWeese TL, Drake CG. Radiation and checkpoint blockade immunotherapy: radiosensitisation and potential mechanisms of synergy. Lancet Oncol. 2015 Oct; 16(13):e498-509.

Siva S, Callahan J, MacManus MP, Martin O, Hicks RJ, Ball DL. Abscopal [corrected] effects after conventional and stereotactic lung irradiation of non-small-cell lung cancer. J Thorac Oncol. 2013 Aug; 8(8):e71-2.

Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma. 2014;2189–2199.

Socinski MA, Jotte RM, Cappuzzo F, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. N Engl J Med. 2018 Jun 14;378(24):2288-2301.

Spranger S, Spaapen RM, Zha Y, et al. Up-regulation of PD-L1, IDO, and T(regs) in the melanoma tumor microenvironment is driven by CD8(+) T cells. Sci Transl Med. 2013;5:200ra116.

Sucker A, Zhao F, Real B, et al. Genetic evolution of T-cell resistance in the course of melanoma progression. Clin Cancer Res. 2014;20:6593-604.

Thomas A, Vilimas R, Trindade C, et al. Durvalumab in combination with olaparib in patients with relapsed SCLC: results from a Phase II study. J Thoracic Oncol 2019:14(8):1447-1457.

Upadhaya S, Hubbard-Lucey VM, and Yu JX. Immuno-oncology drug development forges on despite COVID-19. Nature Reviews Drug Discovery. doi: 10.1038/d41573-020-00166-11.

Uyttenhove C, Pilotte L, The´ate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med 2003;9:1269.

Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science. 2015;350(6257):207–211.

Willingham SB, Ho PY, Hotson A, Hill C, Piccione EC, Hsieh J, et al.A2AR antagonism with CPI-444 induces antitumor responses and augments efficacy to antiePD-(L)1 and antieCTLA-4 in preclinical models. Cancer Immunol Res 2018;6. 1136 LP e 1149.

Woo S-R, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res 2012;72:917e27

Yan Y, Kumar AB, Finnes H, et al. Combining Immune Checkpoint Inhibitors With Conventional Cancer Therapy. Front Immunol. 2018; 9: 1739.

Yau T, Kang YK, Kim TY, et al. Nivolumab (NIVO) + ipilimumab (IPI) combination therapy in patients (pts) with advanced hepatocellular carcinoma (aHCC): Results from CheckMate 040. J Clin Oncol (2019) : 37(Suppl 15): abstract 4012.

Zaretsky JM, Harcia-Diaz A, Shin DS, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med. 2016;375:819-29.

Zhu Y, Knolhoff BL, Meyer MA, Nywening TM, West BL, Luo J, et al. CSF1/CSF1R blockade Reprograms tumor infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. Cancer Res 2014;74. 5057 LP e 5069.

Zitvogel L, Galluzzi L, Smyth MJ, Kroemer G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. Immunity (2013) 39(1):74–88.10.1016/j.immuni.2013.06.014.

Short author biography

Oana Draghiciu, PhD, is a medical writer at CATO SMS, where she develops clinical trial-related documentation in the oncology and immuno-oncology fields. After obtaining her PhD in immuno-oncology at The University Medical Center Groningen (The Netherlands), she worked as a scientist-clinical pharmacist at Sanquin Research in Amsterdam (The Netherlands). Oana has over five years of experience in publication/development and regulatory medical writing in oncology, immuno-oncology and hematology, and a background in pharmaceutical sciences. Email: oana.draghiciu@cato-sms.com

Inka Pawlitzky, PhD, PhD, is director of oncology drug development affairs at CATO SMS, where she provides expert strategic advice on the development of drugs and diagnostics specifically in the field of oncology and immunology. Inka obtained her PhD in immunology at Tufts Medical School (US) Before joining CATO SMS, she conducted postdoctoral fellowships at the Max-Planck Institute for Immunobiology and Epigenetics (Germany) and the Netherlands Cancer Institute (The Netherlands) and held the position of senior scientist at a global molecular diagnostics company. Email: inka.pawlitzky@cato-sms.com

Appendix A – Table 1. Overview of approved ICIs, alone or in combination therapies

Drug	MoA	Initial approval		Approved indications* (single agent)		Approval of combination		Approved indications* (combination therapies)	
		FDA	EMA	FDA	EMA	FDA	EMA	FDA	EMA
Pembrolizumab	Anti-PD-1 mAbs	Sep 2014	May 2015	• Unresectable/metastatic melanoma • NSCLC • HNSCC • Relapsed/refractory classical Hodgkin lymphoma • Locally advanced/metastatic urothelial carcinoma • RCC • Relapsed/refractory PMBCL • MSI-H or dMMR cancer • Gastric cancer/Gastroesophageal junction adenocarcinoma • Esophageal cancer • Cervical cancer • Hepatocellular carcinoma • Merkel cell carcinoma • BCG-high risk non-muscle invasive bladder cancer with carcinoma in situ • Metastatic SCLC • Adult and pediatric TMB-H cancer • Recurrent/metastatic cutaneous squamous cell carcinoma	• Unresectable/metastatic or Stage III melanoma • Metastatic NSCLC • Metastatic/unresectable HNSCC • Relapsed/refractory classical Hodgkin lymphoma • Locally advanced/metastatic urothelial carcinoma • Metastatic/unresectable HNSCC	• Squamous NSCLC: May 2017 • Non-squamous NSCLC with no EGFR/ALK mutations: Oct 2018 • HNSCC: Jun 2019 • RCC: Jun 2019 • Endometrial carcinoma: Sep 2019	• Squamous NSCLC: Jan 2019 • Non-squamous NSCLC with no GFR/ALK mutations: Oct 2018 • HNSCC: Oct 2019 • RCC: Jul 2019	• Metastatic squamous NSCLC, in combination with carboplatin or paclitaxel/nab-paclitaxel • Metastatic non-squamous NSCLC with no EGFR or ALK genomic tumor aberrations, in combination with pemetrexed and platinum CT • Metastatic or unresectable/recurrent HNSCC, in combination with platinum and FU • Advanced RCC, in combination with axitinib • Advanced endometrial carcinoma that is not MSI-H or dMMR, in combination with lenvatinib	• Metastatic squamous NSCLC, in combination with carboplatin or paclitaxel/nab-paclitaxel • Metastatic non-squamous NSCLC with no EGFR or ALK genomic tumor aberrations, in combination with pemetrexed and platinum CT • Metastatic/unresectable HNSCC, in combination with platinum and FU • Advanced RCC, in combination with axitinib
Nivolumab		Dec 2014	Jun 2015	• Unresectable/metastatic melanoma • Metastatic NSCLC • Advanced RCC • Classical Hodgkin lymphoma • Recurrent/metastatic HNSCC • Locally advanced urothelial carcinoma • MSI-H or dMMR CRC • HCC • Recurrent/metastatic SCLC	• Unresectable/metastatic melanoma • Locally advanced/metastatic NSCLC • Advanced, previously treated RCC • Classical Hodgkin lymphoma • Recurrent/metastatic HNSCC • Locally advanced unresectable/metastatic urothelial carcinoma	• Melanoma: Sep 2015 • RCC: Apr 2018 • HCC: Mar 2020 • CRC: Jul 2018	• Melanoma: Apr 2018 • RCC: Nov 2018	• Unresectable/metastatic melanoma, in combination with ipilimumab • Intermediate or poor risk previously untreated advanced RCC, in combination with ipilimumab • HCC, in combination with ipilimumab • MSI-H or dMMR metastatic CRC, in combination with ipilimumab	• Unresectable/metastatic melanoma, in combination with ipilimumab • Advanced, untreated RCC, in combination with ipilimumab

Atezolizumab	Anti-PD-L1 mAbs	May 2016	Jul 2017	• Locally advanced/metastatic urothelial carcinoma • Metastatic NSCLC	• Locally advanced/metastatic urothelial carcinoma • Locally advanced/metastatic NSCLC	• NSCLC: Dec 2018 • TNBC: Mar 2019 • SCLC: Mar 2019	• TNBC: Jun 2019 • NSCLC: Jul 2019 • SCLC: Jul 2019	• Metastatic non-squamous NSCLC with no EGFR or ALK genomic tumor aberrations, in combination with bevacizumab, paclitaxel, and carboplatin or with nab-paclitaxel and carboplatin • Unresectable locally advanced/metastatic TNBC, in combination with nab-paclitaxel • Extensive-stage SCLC, in combination with carboplatin and etoposide	• Unresectable locally advanced/metastatic TNBC, in combination with nab-paclitaxel • Metastatic non-squamous NSCLC with no EGFR or ALK genomic tumor aberrations, in combination with bevacizumab, paclitaxel, and carboplatin • Extensive-stage SCLC, in combination with carboplatin and etoposide
Avelumab		Mar 2017	Sep 2017	• Merkel cell carcinoma • Locally advanced/metastatic urothelial carcinoma	• Metastatic Merkel cell carcinoma	May 2019	Oct 2019	• Advanced RCC, in combination with axitinib	
Durvalumab		May 2017	Sep 2018	• Locally advanced/metastatic urothelial carcinoma • Unresectable Stage III NSCLC	• Locally advanced, unresectable NSCLC	Mar 2020	-	• Extensive-stage SCLC, in combination with etoposide and either carboplatin or cisplatin	-
Cemiplimab		Sep 2018	Jun 2019	• Metastatic/locally advanced cutaneous squamous cell carcinoma		-		-	

Ipilimumab	Anti-CTLA4 mAb	Mar 2011	Jul 2011	• Unresectable/metastatic melanoma • Cutaneous melanoma	• Unresectable/metastatic melanoma (adults and adolescents ≥12 years)	• Melanoma: Sep 2015 • RCC: Apr 2018 • HCC: Mar 2020 • CRC: Jul 2018	• Melanoma: Apr 2018 • RCC: Nov 2018	• Unresectable/metastatic melanoma, in combination with nivolumab • Intermediate or poor risk previously untreated advanced RCC, in combination with nivolumab • MSI-H or dMMR metastatic CRC, in combination with nivolumab • HCC, in combination with nivolumab	• Unresectable/metastatic melanoma (adults), in combination with nivolumab • Intermediate/poor-risk advanced RCC, in combination with nivolumab

*These represent extensions of the original indication, classified as supplements to the original approval.

Abbreviations: MoA: mechanism of action; ALK: anaplastic lymphoma kinase; BCG: Bacille Calmette-Guerin; CRC: colorectal cancer; CT: chemotherapy; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; dMMR: mismatch repair deficient; EGFR: endothelial growth factor receptor; EMA: European Medicines Agency; FDA: Food and Drug Administration; FU: fluorouracil; HCC: hepatocellular carcinoma; HNSCC: head and neck squamous cell carcinoma; MSI-H: microsatellite instability high; NSCLC: non-small cell lung cancer; PD-1: programmed cell death protein 1; PD-L1: programmed death ligand 1; PMBCL: primary mediastinal B-cell lymphoma; RCC: renal cell carcinoma; SCLC: small cell lung cancer; TMB-H: tumor mutational burden-high; TNBC: triple-negative breast cancer.

Table 2. Next generation checkpoint inhibitors in combination therapies assessed in clinical trials.

Drug group	Target	Compound	Combination therapy	Tumor type	Selected clinical trials (phase and ID)
Lymphoid inhibitors	**LAG-3**	Relatlimab	• Anti-PD-1 (nivolumab) ± anti-CTLA-4 (ipilimumab) • For the NCT04062656 trial: Anti-PD-1 (nivolumab) + anti-CTLA-4 + chemotherapy (oxaliplatin, docetaxel, and 5-FU)	• Solid tumors • NSCLC • Metastatic melanoma • Head and neck cancer • Gastric cancer • Colorectal cancer • AML	• Phase I/II, NCT01968109 (Ascierto et al, 2017) • Phase II, NCT04205552* • Phase II, NCT03743766* • Phase II, NCT04080804* Phase II, NCT04326257*^ • Phase II, NCT04062656* Phase II, EudraCT-2018-000383-28*^ • Phase II, NCT03867799* • EudraCT-2018-002939-21*
	TIGIT	Tiragolumab	• Anti-PD-L1 (atezolizumab) • Anti-PD-L1 (atezolizumab) + chemotherapy (carboplatin an etoposide)	• NSCLC • SCLC	• Phase III, NCT04294810* • Phase III, NCT04256421*
	TIM-3	Sym023	• Anti-PD-1 (Sym021)	• Solid tumors, lymphoma	• Phase I, NCT03311412
		TSR-022	• Anti-PD-1 (TSR-042)	• Solid tumors • Hepatocellular carcinoma • Melanoma	• Phase I, NCT02817633 • Phase II, NCT03680508* • Phase II, NCT04139902*
		MBG453	• Anti-PD-1 (PDR001) • Decitabine • Hypomethylating agents • Azacitidine	• Solid tumors • Myelodysplastic syndromes	• Phase I/II, NCT02608268 • Phase Ib, NCT030066648 (Borate et al, 2019) Phase II, NCT03946670* Phase III, NCT04266301*
		LY3321367	• Anti-PD-1 (LY3300054)	• Solid tumors	• Phase I, NCT03099109
	CD73	Oleclumab	• Anti-CTLA-4 (durvalumab)	• Colorectal cancer • Prostate cancer • Breast cancer • NSCLC	• Phase I, NCT02503774 (Overman et al, 2018) • Phase II, NCT04089553* • Phase II, NCT03875573* • Phase II, NCT03493581 Phase II, NCT03794544*

Non-lymphoid inhibitors	**IDO1**	Indoximod	• Cellular therapy (sipuleucel-T) • Chemotherapy (gemcitabine, nab-paclitaxel) • Chemotherapy and radiation	• Metastatic prostate cancer • Glioblastoma and glioma	• Phase II, NCT01560923 (Jha et al, 2017) Phase II, NCT02077881 (Bahary et al, 2016) • Phase II, NCT04049669*
		Epacadostat	• Anti-PD-1 (pembrolizumab) • Anti-VEGF (bevacizumab) + radiation	• Melanoma • Head and neck cancer • Glioblastoma and glioma	• Phase III, NCT02752074 (Long et al, 2018) • Phase II, NCT03823131* • Phase II, NCT03532295*
		BMS-986205	• Anti-PD-1 (nivolumab) or anti-PD-1 (nivolumab) + anti-CTLA-4 (ipilimumab)	• Solid tumors • Endometrial cancer	• Phase I/II, NCT02658890 (Luke et al, 2017) • Phase II, NCT04106414*
	CSF-1R	Emactuzumab	• Chemotherapy (paclitaxel) • Chemotherapy (paclitaxel) ± VEGF-targeted therapy (bevacizumab)	• Solid tumors • Ovarian cancer	• Phase I, NCT01494688 (Gomez-Roca et al, 2019) • Phase II, NCT02923739

*Initiated between Jan 1st, 2019 and Jun 30th, 2020.
^Relatlimab combined with nivolumab in association with ipilimumab.

Abbreviations: 5-FU: 5-fuorouracil; AML: acute myeloid leukemia; CD73: cluster of differentiation 73; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; CSF-1R : colony stimulating factor 1 receptor; IDO1: indoleamine 2,3-dioxygenase 1; ITIM: immunoreceptor tyrosine-based inhibitory motif; LAG-3: lymphocyte-activation gene 3; NSCLC: non-small cell lung cancer; PD-1: programmed cell death protein 1; PD-L1: programmed death ligand 1; TIGIT: T cell immunoreceptor with immunoglobulin and ITIM domains; TIM-3: T-cell immunoglobulin and mucin-domain containing-3; SCLC: small cell lung cancer.