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REVIEW ARTICLE

The New Immunotherapy Combinations in the Treatment of Advanced Non-Small Cell Lung Cancer: Reality and Perspectives

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Abstract: ***Background***: In the recent years, immunotherapeutics and specifically immune-checkpoints inhibitors have marked a significant shift in the diagnostic and therapeutic algorithm of Non-Small Cell Lung Cancer (NSCLC), allowing us to use immunotherapeutics alone or combined with chemotherapy for a great subset of patients. However, new interesting approaches are being presently investigated, markedly immunotherapy combinations, that is, the use of two or more immunotherapeutics combined.

Methods: In particular, the combination of anti-PD-1 nivolumab and anti-CTLA-4 ipilimumab has already provided groundbreaking positive results in the advanced NSCLC and other combinations are currently under investigation.

Results: Therefore, this paper aims to provide a comprehensive state-of-the-art review about immunotherapy combination, along with suggestions about future directions. A comprehensive literature search was carried out to identify eligible studies from MEDLINE/PubMed and ClinicalTrials.gov.

Conclusion: Nivolumab plus ipilimumab represent the most promising immunotherapy combination for the treatment of advanced NSCLC patients; safety, tolerability and efficacy of new immunotherapeutics (in monotherapy and in immunotherapy combinations) must be further assessed in future studies.

Keywords: NSCLC, immunotherapy, combination therapy, immune modulators, TMB, LAG-3, OX40, IDO1.

1. INTRODUCTION

1.1. Advanced NSCLC and Immunotherapy

To date, lung cancer - more specifically Non-Small Cell Lung Cancer (NSCLC) that accounts for about 85% of all lung cancer cases - is both the most frequently diagnosed and deadliest cancer among males, while it is the third most frequently diagnosed yet the deadliest one among females; when we consider both sexes, it is both the most common malignancy and the leading cause of death for cancer, accounting for circa 12% of all cancers (approximately 2,000,000 cases/year) [1].

In this context, since nivolumab FDA (Food and Drug Administration)-approval in early 2015, immunotherapy *i.e.* employing substances to enhance patient's immune system response to cancer - more specifically in the form of ICIs (Immune Checkpoint Inhibitors) has marked one of the most significant shifts in the diagnostic and therapeutic paradigms of advanced NSCLC (IIIB/IV TNM stage, accounting for 80% of all NSCLC diagnoses), managing to grant unprecedented results and becoming in few years the standard of care in different settings, according to the most recent international guidelines [2].

1.2. Preclinical Rationale for Immunotherapy in Solid Tumors

In order to evade immunosurveillance and more specifically to escape T-cell-mediated cytolysis, cells from different solid tumors - notably NSCLC cells among them - manage to create an immunosuppressive micro-environment, exploiting to their advantage, a large set of immune-related molecules: surface receptors, ligands and enzymes. Keeping in mind this principle, to be able to understand and precisely modulate the mechanisms underlying tumor microenvironment immunity stimulation and immunosuppression is presently one of the biggest challenges of pulmonary immune-oncology [3]. More specifically, some of these molecules have increasingly gained attention over the last years and currently represent the most promising targets in this field.

1.2.1. Negative T-cells Immune-checkpoint Regulators

1.2.1.1. PD-L1-PD-1

PD-L1 (Programmed Death Ligand-1 also known as CD274), is a member of the B7 protein family physiologi-

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cally expressed on APCs surface whose immunosuppressive activity depends on binding with its receptor Programmed Death Protein 1 (PD-1 also known as CD279), *i.e.* a transmembrane receptor mainly found on T-cells. In fact, the PD-L1-PD1 binding (that mainly takes place in peripheral tissues) causes T-cells inactivation and apoptosis [4-6]; however, also cancer cells have appropriated this mechanism, (over)expressing PD-L1 on their surface, in order to evade immunosurveillance and to escape T-cell-mediated cytolysis [7-9].

Therefore, the PD-L1 - PD-1 pathway has become one of the main targets of cancer immunotherapy, in fact, blocking this immune checkpoint through specific monoclonal antibodies targeting either PD-1 or PD-L1, prevents T-cells inactivation and apoptosis and elicits the host's immune response to tumor cells [10, 11].

The only ICIs currently FDA and EMA-approved and defined as the standard of care for metastatic NSCLC (nivolumab, pembrolizumab and atezolizumab) employ this mechanism of action, blocking either PD-1 (nivolumab and pembrolizumab) or PD-L1 (atezolizumab) [12-14].

1.2.1.2. CTLA-4/CD28-B7-1/2

Physiologically, in order to be activated and to proliferate, T-cells need at least two different signals: TCR (T-cell surface receptor)-MHC (APCs surface ligand) binding, that qualifies as the principal activation signal and a costimulatory signal and CD28 (T-cell surface receptor)-B7-1/2 (APCs surface ligand) binding, that acts as a costimulatory signal. On the other hand, CTLA-4 (CD 152) is a CD28 homolog with superior binding affinity to B7, but, just like PD-1, it is a negative regulator of the T-lymphocyte activation and competes (principally in lymph nodes) with CD28 for binding with B7, therefore, CTLA-4-(over)expression on the surface of cancer cells and/or T-cells can avert the CD28-B7-1/2 costimulatory signal, leading to T-cells anergy and apoptosis [15-18]. Similar to anti-PD-1/PD-L1 drugs, preventing CTLA-4 from binding with B7 through a monoclonal antibody (anti CTLA-4 mAbs), T-lymphocyte anergy and apoptosis could be prevented, also enhancing the CD28-B7-1/2 binding ratio and thus improving T-cell activation and proliferation [19, 20].

1.2.1.3. LAG-3-MHC II

LAG-3 (Lymphocyte activation gene-3, also known as CD223) is another negative modulator of the T-cells activation, unlike PD-L1 and CTLA-4, however, it is found only on the surface of activated T-lymphocytes (principally) [21, 22], NK cells, B cells and plasmacytoid DC, and exerts its function by binding to its main ligand: class II MHC, expressed on the surface of APCs. Hence, the blockade of LAG-3 pathway through anti-LAG-3 monoclonal antibodies could represent a feasible way of enhancing T-cells activation and expansion [23].

1.2.1.4. TIM-3-TIM Ligands

TIM-3 (T cell immunoglobulin and mucin-domain containing-3) is a co-inhibitory regulator of T-cells activity expressed on the surface of T-lymphocytes (mainly) and innate immune cells, whose action is carried out by ligation with its (still partially unknown) ligands found on the surface of APCs (galectin-9, Caecam1, phosphatidylserine and HMGB1 appear to be among the most probable ones) [24]; therefore, the use of anti-TIM-3 monoclonal antibodies to block its pathway could be a promising approach to augment the T-lymphocytes activity and thus, ultimately, the killing of cancer cells [25].

1.2.1.5. B7-H3

Like PD-L1, B7-H3 (also known as CD276) is a T-cells activity down-regulator, member of the B7 superfamily, normally expressed on the surface of APCs, that achieves its function by binding its still not clearly identified receptors on the surface of T-lymphocytes [26, 27], nevertheless, cancer cells (NSCLC cells among them) exploit this molecule too, (over)expressing it on their surface, in order to establish an immunosuppressive micro-environment [28]. Consequently, B7-H3 could represent a possible new target for immune-oncology treatments [29, 30].

1.2.1.6. VISTA

V-domain Ig-containing Suppressor of T-cell Activation (VISTA) is a negative modulator of T-lymphocytes activity and proliferation expressing some unusual features; in fact, it can be found both on T-cells surface, acting as a receptor and on APCs cells surface, acting as a ligand; currently, its ligand/receptor cannot be described, but it is supposed that VISTA exerts its T-suppressive action through this binding [31, 32]. In this sense, the inhibitory agents targeting VISTA in order to prevent its binding seem to be another possible way of improving the patient's immune response to tumor cells [33].

1.2.2. Negative NK Cells Immune-checkpoint Regulators

1.2.2.1. KIR-MHC I

Killer Ig-like Receptors (KIRs) are inhibitory receptors found on the surface of NK cells, that block the NK cells function and activation after ligation with specific MHC class I molecules. In fact, both KIRs and MHC class I molecules are associated with considerable polymorphisms and mismatched KIR-MHC I association or the lack of MHC class I molecules lead to MHC I-presenting cell cytolysis [34]. Given that KIRs expression (alongside with other NK cells defects) seems to be increased in cancer patients [35], the use of anti-KIR monoclonal antibodies could enable us to stimulate NK cells-dependent cancer killing [36, 37].

1.2.3. Positive T-cells Immune-checkpoint Regulators

1.2.3.1. 4-1BB-4-1BB-L

4-1BB (also known as CD137 or TNFRSF9) is a member of the tumor Necrosis Factor Receptor (TNFR) superfamily located principally on the surface of activated T-cells, whose ligand 4-1BB-L is expressed on the surface of APCs. Upon binding with its ligand, 4-1BB acts as a co-stimulatory agent (TCR-MHC II being the principal activation signal), inducing pro-proliferative, pro-inflammatory, pro-cytotoxic and pro-cytolytic signals in T-cells [38, 39]. Considering these data, the stimulation of 4-1BB (and thus of T-cells-mediated immunity) through agonistic monoclonal antibodies mimick-

ing 4-1BB-L function could hold a potential role in cancer immunotherapy [40, 41].

1.2.3.2. OX40-0X40-L

OX40 (also known as CD134 or TNFRSF4) is another member of the TNFR superfamily, notably found on the surface of activated T-cells (but also on the surface of other lymphoid and non-lymphoid cells), that provides activating co-stimulatory signals to T-cells after its ligation with OX40-L (found on the surface of APCs), that ultimately leads to augmented proliferation, clonal expansion, survival and cytokine production. Moreover, OX40-OX40-L ligation partially suppresses Treg-cells activation, further enhancing the above-mentioned process [42, 43]. Agonistic anti-OX40 monoclonal antibodies are currently under investigation in order to exploit this pathway (and hence to boost immune cells activity against tumor cells) in different cancer immunotherapy settings, advanced NSCLC among them [44].

1.2.3.3. GITR-GITRL and CD27-CD70

GITR (Glucocorticoid-Induced TNFR-related protein, also known as TNFRSF18) and CD27 share a lot of features with OX40. In fact, both GITR and CD27 represent members of the TNFR family and are expressed on the surface of activated T-cells and the interaction with their ligands (GITRL and CD70, respectively) expressed on APCs triggers co-stimulatory signals that boost T-effectors activity, while suppressing Treg-cells functions [45, 46]. These data, thus, provide the basis for a potential anti-GITR/CD27 agonistic mAbs-based treatment [47, 48].

1.2.4. Other Tumor Microenvironment Immunosuppressive Molecules

1.2.4.1. IDO1

Not only can cancer cells exploit immunosuppressive receptors and ligands to evade immunosurveillance, they can also employ immunosuppressive enzymes expressed in their microenvironment. IDO1 (Indoleamine 2, 3-dioxygenases) is a member of the family of tryptophan-catabolic enzymes - responsible for catalyzing the conversion of tryptophan into kynurenine - along with IDO2 and Tryptophan 2, 3-Dioxygenase (TDO), however, IDO2 and TDO are less expressed and much more tissue-specific, qualifying IDO1 as the key member. Physiologically, IDO1 contributes to the innate immune mechanism, due to the anti-pathogen action of tryptophan metabolites and to immune tolerability, considering the fact that tryptophan metabolites exert immunosuppressive activity through the inhibition of T-effectors and NK cells functions and through the enhancement of Treg cells, DCs and MDSCs (myeloid-derived suppressor cells) activity [49, 50]. However, in more than 50% of cancers, tumor cells (NSCLC cells among them [51, 52]) overexpress IDO1, contributing to the creation of an immunosuppressive microenvironment that ultimately leads to cancer cells immune escape; the implementation of IDO1 inhibitors, could represent a feasible way of (partially) preventing immunosuppression [53-55].

1.2.4.2. Extracellular Adenosine

It is well established that, while in a physiological setting, extracellular levels of adenosine are typically low, due to reduced extracellular ATP and adenosine production and to cellular uptake mechanisms, hypoxia, tissue damage and high cell turnover (notably associated with tumor microenvironment) can significantly increase extracellular ATP and adenosine levels [56]. Extracellular adenosine, moreover, upon interaction with its receptors A1, A2A (also known as ADORA2A, presenting the highest affinity and currently considered the major receptor), A2B and A3 - widely expressed on immune and endothelial cells - triggers immunosuppressive signals, that lead to intensified production of immunosuppressive cytokines, up-regulation of PD-L1 and CTLA-4 axes, inhibition of T-effectors activity and to the enhancement of Treg cells proliferation and functions [57]. In light of these data, anti-ADORA2A treatment could hold a potential role in cancer immunotherapy [58].

1.2.4.3. TGF-β

TGF-β (transforming growth factor- β) is a regulatory polypeptide with some peculiar features, in fact, while physiologically after ligation with its receptors, it exerts both anti-tumor and immunosuppressive pro-tolerance activity, the former through apoptosis, cell-cycle and differentiation arrest, the latter through the suppression of T-effectors, NK cells and DCs functions and through the boost of Tregs functions; the TGF-β pathway can be dysregulated as well, markedly in cancer. In this scenario, TGF- β acts as a pro-oncogenic, promoting the establishment of an immunosuppressive micro-environment and favoring Epithelial-Mesenchymal Transition (EMT), cell survival, proliferation and angiogenesis [59-61]. TGF-β inhibitors are presently under investigation in order to assess their suitability in various neoplasms, metastatic NSCLC amongst them [62, 63].

1.3. Rationale for Immunotherapy Combinations in Advanced NSCLC

Just like any other treatment, immunotherapy has to face two major hurdles: response to therapy and resistance to therapy. In fact, as the above-mentioned data have shown, there is an extensive set of immune-related molecules underlying the creation of the immunosuppressive tumor microenvironment, therefore immunotherapy administered in monotherapy, targeting just one specific pathway, cannot grant in the majority of cases, neither great RRs (response rates) nor long term response due to both cancer cells intrinsic exploitation of multiple pathways to evade immune-mediated killing and rapid adaptation under selective pressure to the same end [64, 65].

In fact, taking into account specifically NSCLC and considering data from the trials that led to the approval of nivolumab, pembrolizumab and atezolizumab in the second-line setting after chemotherapy failure, it is worth to mention that the RRs were 19%/ 20%, 18% and 14%, respectively, while mOS (median overall survivals) were 12.2 months/9.2 months, 10.4 months and 13.8 months, respectively [66-69]. Therefore, immunotherapy combinations (*i.e.* the use of two or more immunotherapeutics combined), targeting multiple pathways at once, could represent an effective therapeutic solution to enhance response to treatment and to delay the establishment of acquired resistance mechanisms in NSCLC [70-72].

1.4. Current Landscape of Immunotherapy Combinations in Advanced NSCLC: Anti PD-1/PD-L1 + Anti CTLA-4

1.4.1. Durvalumab + Tremelimumab

One of the first immunotherapy combinations proposed in IIIB/IV TNM stage NSCLC consisted of an anti-PD-L1 monoclonal antibody (durvalumab) plus an anti-CTLA-4 monoclonal antibody (tremelimumab). After initial promising results in a phase Ib trial in which durvalumab at a dose of 20 mg/kg plus tremelimumab at a dose of 1 mg/kg every 4 weeks showed antitumor activity irrespectively of PD-L1 expression and a good safety and tolerability profile [73], it was further investigated with the same dosage schedule in three phase III trials: NEPTUNE, MYSTIC and ARCTIC. In the NEPTUNE study, 960 advanced NSCLC patients without driver gene mutations were randomly assigned (1:1) to receive durvalumab + tremelimumab or SoC (Standard of Care) chemotherapy in a first-line setting, with OS as the primary endpoint, in the MYSTIC trial, 1850 advanced NSCLC patients without driver gene mutations were randomly assigned (1:1:1) to receive durvalumab ± tremelimumab or SoC chemotherapy in a first-line setting, with PFS and OS as primary endpoints. In the ARCTIC study, 900 advanced NSCLC patients without driver gene mutations that had already progressed on ≥ 2 previous lines of therapy (including one platinum-based one) were randomly assigned (1:1) to receive ± tremelimumab or SoC chemotherapy, PFS and OS were the primary endpoints [74-76]. Unfortunately, none of these trials managed to meet its primary endpoints, failing to show better performances than SoC chemotherapy [77].

1.4.2. Pembrolizumab + Ipilimumab

Another combinational approach is represented by pembrolizumab (anti PD-1) plus ipilimumab (anti CTLA-4), this doublet was assessed in the cohorts D and H of phase I/II study, the KEYNOTE-021 trial, in which it was administered every three weeks (pembrolizumab 2 mg/kg + ipilimumab 1 mg/kg) to 45 advanced NSCLC-affected patients without driver gene mutations that had already received ≥ 1 line of therapy. This association showed promising results that proved to be irrespective of PD-L1 expression: ORR: 24%, DOR (duration of response): 14 months, mPFS: 6 months, mOS: 17 months, alongside with a favorable safety and tolerability profile: 67% of treated patients reported TRAEs (Treatment-Related Adverse Events), 24% of which were G3-G5 [78]. Due to these results, this combination (pembrolizumab at a dose of 200 mg every three weeks plus ipilimumab at a dose of 1 mg/kg every six weeks) is presently

Table 1. Available data on anti-PD-1/PD-L1 + anti-CTLA-4 immunotherapy combinations in advanced NSCLC.

Trial	Phase and Combination	Results†
NCT02000947	Phase Ib Durvalumab + Tremelimumab in naive patients	ORR: 23% G3-G5 TRAEs: 36%
KEYNOTE-021 (cohorts D and H)	Phase I/II Pembrolizumab + ipilimumab after ≥ 1 line of therapy	ORR: 24% DOR: 14 months mPFS: 6 months mOS: 17 months Any grade TRAEs: 67% G3-G5 TRAEs:24%
CheckMate 012	Phase I Nivolumab + ipilimumab in naive patients	ORR: 47% G3-4 TRAEs: 37% mPFS: 8.1 months (Schedule 1) ORR: 38% G3-4 TRAEs: 33% mPFS: 3.9 months (Schedule 2)
CheckMate 227	Phase III Nivolumab + ipilimumab in naive patients (TMB ≥ 10 mutations per megabase)	ORR: 45.3% 1-year PFS rate: 42.6% mPFS: 7.2 months HR for PD or death: 0.58 G3-4 TRAEs rate: 31.2% HR: 0.77 mOS: 23.03 months
CheckMate 227	Phase III Nivolumab + ipilimumab in naive patients (TMB < 10 mutations per megabase)	HR: 0.78 mOS: 16.20 months

†ORR: Objective Response Rate, DOR: Duration of Response, mPFS: median Progression-Free Survival, mOS: median Overall Survival, TRAEs: Treatment-Related Adverse Events according to the CTCAE, HR: Hazard Ratio, PD: Progression of disease

Table 2. Phase I/II trials of new immunotherapeutics + anti-PD-1/PD-L1/CTLA-4 ICIs or + other new immunotherapeutics in advanced NSCLC.

ClinicalTrials.gov Identifier	Design†	Phase
NCT02460224	LAG525 (anti LAG-3 mAb) ± PDR001 (anti PD-1 ICI)	I/II
NCT03250832	TSR-033 (anti LAG-3 mAb) ± Anti PD-1 ICI	I
NCT01968109	BMS-986016 (anti LAG-3 mAb) ± Nivolumab (anti PD-1 ICI)	I/II
NCT03005782	REGN3767 (anti LAG-3 mAb) ± REGN2810 (anti PD-1 ICI)	I
NCT02966548	BMS-986016 (anti LAG-3 mAb) ± Nivolumab (anti PD-1 ICI)	I
NCT03459222	BMS-986016 (anti LAG-3 mAb) + Nivolumab (anti PD-1 ICI) + BMS-986205 (IDO1 inhibitor) or BMS-986016 (anti LAG-3 mAb) + Nivolumab (anti PD-1 ICI) + Ipilimumab (anti CTLA-4 ICI)	I/II
NCT03099109	LY3321367 (anti TIM-3 mAb) ± LY3300054 (anti PD-L1 ICI)	I
NCT03744468	BGB-A425 (anti TIM-3 mAb) + Tislelizumab (anti PD-1 ICI)	I/II
NCT02608268	MBG453 (anti TIM-3 mAb) ± PDR001 (anti PD-1 ICI)	I/II
NCT02817633	TSR-022 (anti TIM-3 mAb) ± Anti-PD-1 ICI	I
NCT02475213	MGA271 (anti B7-H3 mAb) + Pembrolizumab (anti PD-1 ICI)	I
NCT02381314	MGA271 (anti B7-H3 mAb) + Ipilimumab (anti CTLA-4 ICI)	I
NCT03406949	MGD009 (anti B7-H3 mAb) + MGA012 (anti PD-1 ICI)	I
NCT03729596	MGC018 (anti-B7-H3 mAb) ± MGA012 (anti PD-1 ICI)	I/II
NCT01750580	BMS-986015 (anti-KIR mAb) + Ipilimumab (anti CTLA-4 ICI)	I
NCT01714739	BMS-986015 (anti-KIR mAb) + Nivolumab (anti PD-1 ICI) ± Ipilimumab (anti CTLA-4 ICI)	I/II
NCT03203876	BMS-986015 (anti-KIR mAb) + Nivolumab (anti PD-1 ICI) ± Ipilimumab (anti CTLA-4 ICI)	I
NCT02554812	PF-05082566 (agonistic anti-4-1BB mAb) + Avelumab (anti PD-L1 ICI) or PF-04518600 (agonistic anti-OX-40 mAb) + Avelumab (anti PD-L1 ICI) or PF-05082566 (agonistic anti-4-1BB mAb) + PF-04518600 (agonistic anti-OX-40 mAb) + Avelumab (anti PD-L1 ICI)	I/II
NCT02315066	PF-05082566 (agonistic anti-4-1BB mAb) + PF-04518600 (agonistic anti-OX-40 mAb)	I
NCT02528357	GSK3174998 (agonistic anti-OX-40 mAb) ± Pembrolizumab (anti PD-1 ICI)	I
NCT02410512	MOXR0916 (agonistic anti-OX-40 mAb) + Atezolizumab (anti PD-L1 ICI)	I
NCT02221960	MEDI6383 (agonistic anti-OX-40 mAb) ± MEDI4736 (anti PD-L1 ICI)	I
NCT02740270	GWN323 (agonistic anti-GITR mAb) ± PDR001 (anti-PD-1 ICI)	I
NCT03126110	INCAGN01876 (agonistic anti-GITR mAb) + Nivolumab (anti PD-1 ICI) or INCAGN01876 (agonistic anti-GITR mAb) + Ipilimumab (anti CTLA-4 ICI) or INCAGN01876 (agonistic anti-GITR mAb) + Nivolumab (anti PD-1 ICI) + Ipilimumab (anti CTLA-4 ICI)	I/II
NCT02335918	Varlilumab (agonistic anti-CD27 mAb) + Nivolumab (anti PD-1 ICI)	I/II
NCT02559492	INCB024360 (IDO1 inhibitor) + Pembrolizumab (anti PD-1 ICI)	II
NCT03335540	BMS-986015 (anti-KIR mAb) + Nivolumab (anti PD-1 ICI) or BMS-986016 (anti LAG-3 mAb) + Nivolumab (anti PD-1 ICI) or BMS-986156 agonistic anti-GITR mAb) + Nivolumab (anti PD-1 ICI) or BMS-986205 (IDO1 inhibitor) + Nivolumab (anti PD-1 ICI)	I

(Table 2) contd….

ClinicalTrials.gov Identifier	Design†	Phase
NCT03491631	SHR9146 (IDO1 inhibitor) + SHR-1210 (anti PD-1 ICI)	I
NCT03343613	LY3381916 (IDO1 inhibitor) ± LY3300054 (anti PD-L1 ICI)	I
NCT03207867	NIR178 (ADORA2A inhibitor) + PDR001 (anti PD-1 ICI)	II
NCT02403193	PBF-509 (ADORA2A inhibitor) ± PDR001 (anti PD-1 ICI)	I/II
NCT02655822	CPI-444 (ADORA2A inhibitor) ± Atezolizumab (anti PD-L1 ICI)	I
NCT03629756	AB928 (ADORA2A inhibitor) + AB122 (anti PD-1 ICI)	I
NCT02740985	AZD4635 (ADORA2A inhibitor) ± Durvalumab (anti PD-L1 ICI)	I
NCT02423343	LY2157299 (TGF- β inhibitor) + Nivolumab (anti PD-1 ICI)	I/II
NCT02937272	LY3200882 (TGF- β inhibitor) ± LY3300054 (anti PD-L1 ICI)	I

†mAb: monoclonal Antibody; ICI: Immune Checkpoint Inhibitor; LAG-3: Lymphocyte activation gene-3; TIM-3: T cell immunoglobulin and mucin-domain containing-3; KIR: Killer Ig-like receptors; GITR: glucocorticoid‑induced TNFR; IDO1: Indoleamine 2, 3-dioxygenases; TGF-β: transforming growth factor- β

being investigated in a phase III study in a first-line setting for PD-L1 positive patients (PD-L1 expression ≥ 50%) [79].

1.4.3. Nivolumab + Ipilimumab

Lastly, nivolumab (anti PD-1) + ipilimumab (anti CTLA-4) is one of the most investigated and interesting combinations currently available for the treatment of advanced NSCLC without driver gene mutations. In fact, this doublet was first assessed in a phase I study as a first-line setting treatment (CheckMate 012), in which it was administered to 77 patients according to two different schedules (38 and 39 patients, respectively): nivolumab 3 mg/kg every 2 weeks + ipilimumab 1 mg/kg every 12 weeks or every 6 weeks, showing encouraging results: ORR: 47% and 38%, respectively and a favorable safety and tolerability profile [80].

In addition, in a groundbreaking recent phase III trial (CheckMate 227), exploiting the data from the CheckMate 568 trial [81], this combination was also evaluated in untreated IV stage NSCLC-affected patients without driver gene mutations but with a high TMB (≥10 mutations per megabase), TMB standing for Tumor Mutational Burden, that is to say the total number of mutations per DNA coding region). One thousand one hundred and eighty-nine patients with a PD-L1 expression of at least 1% were randomly assigned (1:1:1) to receive nivolumab (3 mg/kg every 2 weeks) + ipilimumab (1 mg/kg every 6 weeks), SoC chemotherapy every 3 weeks for up to four cycles, or nivolumab (240 mg every 2 weeks), while 550 patients with a PD-L1 expression of less than 1% were randomly assigned (1:1:1), to receive nivolumab (3 mg/kg every 2 weeks) + ipilimumab (1 mg/kg every 6 weeks), SoC chemotherapy every 3 weeks for up to four cycles, or nivolumab (360 mg) plus SoC chemotherapy every 3 weeks for up to four cycles. As a result, in patients with a high TMB, the nivolumab + ipilimumab combination managed to grant better performances than SoC chemotherapy according to every outcome measure considered, independently of the PD-L1 expression levels: ORR: 45.3% *vs*. 26.9%, 1-year PFS rate: 42.6% vs. 13.2%, mPFS: 7.2 months vs. 5.5%, Hazard Ratio for disease progression or death: 0.58 (P<0.001), G3-4 TRAEs rate: 31.2% *vs*. 36.1% [82]. However, in a recent press release, as requested by the EMA-CHMP (Committee for Medicinal Products for Human Use) in Europe and by the FDA (Food and Drugs Administration) in the USA, the Bristol-Myers Squibb Company provided additional information about the overall survival analyses of the CheckMate-227 trial, involving both the high-TMB (≥10 mutations per megabase) and the low-TMB (<10 mutations per megabase) subgroups of patients. These updated analyses showed that the HRs for OS with nivolumab plus ipilimumab against chemotherapy were comparable between the two subgroups (0.77 and 0.78, respectively) and that the mOS data favored the immune combination over chemotherapy in both these subsets of patients (23.03 months *vs.* 16.72 months and 16.20 months *vs*. 12.42, respectively), seemingly redefining the Tumor Mutational Burden as a prognostic biomarker and qualifying nivolumab + ipilimumab as the most promising immunotherapy combination to date in the treatment of advanced NSCLC, irrespectively of the high/low TMB status and of the PD-L1 expression levels [83] (Table **1**).

1.5. Future Perspectives of Immunotherapy Combinations in Advanced NSCLC: New Immunotherapeutics + Anti-PD-1/PD-L1/CTLA-4 ICIS or + other New Immunotherapeutics

Apart from the well-established role of the PD-1 - PD-L1 and CTLA-4/CD28 - B7-1/2 pathways, the potential role in the therapy of the new immune modulators (negative T-cells immune-checkpoint regulators, negative NK cells immune-checkpoint regulators, positive T-cells immune-checkpoint regulators, other tumor microenvironment immunosuppressive molecules) is practically uncharted. Therefore, the safety, tolerability and efficacy profiles of immunotherapeutics specifically designed for these new targets are currently being investigated in several phase I/II trials, both in monotherapy and in combination (with anti-PD-1/PD-L1/CTLA-4 ICIs or with other new immunotherapeutics) (Table **2**) [84-119].

CONCLUSION

As the above-mentioned data have shown, to date, the nivolumab plus ipilimumab association qualifies as the most promising immunotherapy combination for the treatment of advanced NSCLC patients, being the largest phase III study with the most remarkable results in this field. As regards

new immunotherapeutics, they represent undoubtedly interesting - yet still fully investigational - agents, whose role in therapy (both in monotherapy and in immunotherapy combinations) needs to be further elucidated by ongoing trials.

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CONFLICT OF INTEREST

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REFERENCES

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68(6): 394-424. http://dx.doi.org/10.3322/caac.21492 PMID: 30207593

[2] Planchard D, Popat S, Kerr K, *et al.* . Metastatic non-small cell lung cancer: ESMO Clinical Prac-tice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2018; 29(Supplement_4): iv192-237.

[3] Rocco D, Della Gravara L, Avellino A, *et al.* Immunotherapy as a targeted therapy in non-small cell lung cancer. Transl Cancer Res [in press]. http://dx.doi.org/10.21037/tcr.2018.10.10

[4] Alsaab HO, Sau S, Alzhrani R, *et al.* PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: Mechanism, combinations, and clinical outcome. Front Pharmacol 2017; 8: 561. http://dx.doi.org/10.3389/fphar.2017.00561 PMID: 28878676

[5] Xu-Monette ZY, Zhang M, Li J, Young KH. PD-1/PD-L1 blockade: Have we found the key to unleash the antitumor immune response? Front Immunol 2017; 8: 1597. http://dx.doi.org/10.3389/fimmu.2017.01597 PMID: 29255458

[6] Blank C, Gajewski TF, Mackensen A. Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: Implications for tumor immunotherapy. Cancer Immunol Immunother 2005; 54(4): 307-14. http://dx.doi.org/10.1007/s00262-004-0593-x PMID: 15599732

[7] Hui Y, Boyle TA, Zhou C, *et al.* PD-L1 expression in lung cancer. J Thorac Oncol 2016. http://dx.doi.org/10.1016/j.jtho.2016.04.014

[8] Meyers DE, Bryan PM, Banerji S, Morris DG. Targeting the PD-1/PD-L1 axis for the treatment of non-small-cell lung cancer. Curr Oncol 2018; 25(4): e324-34. http://dx.doi.org/10.3747/co.25.3976 PMID: 30111979

[9] Juneja VR, McGuire KA, Manguso RT, *et al.* PD-L1 on tumor cells is sufficient for immune evasion in immunogenic tumors and inhibits CD8 T cell cytotoxicity. J Exp Med 2017; 214(4): 895-904. http://dx.doi.org/10.1084/jem.20160801 PMID: 28302645

[10] Jain P, Jain C, Velcheti V. Role of immune-checkpoint inhibitors in lung cancer. Ther Adv Respir Dis 2018; 121753465817750075 http://dx.doi.org/10.1177/1753465817750075 PMID: 29385894

[11] Assi HI, Kamphorst AO, Moukalled NM, Ramalingam SS. Immune checkpoint inhibitors in advanced non-small cell lung cancer. Cancer 2018; 124(2): 248-61. http://dx.doi.org/10.1002/cncr.31105 PMID: 29211297

[12] Guo L, Zhang H, Chen B. Nivolumab as programmed death-1 (PD-1) inhibitor for targeted immunotherapy in tumor. J Cancer 2017; 8(3): 410-6. http://dx.doi.org/10.7150/jca.17144 PMID: 28261342

[13] McDermott J, Jimeno A. Pembrolizumab: PD-1 inhibition as a therapeutic strategy in cancer. Drugs Today (Barc) 2015; 51(1): 7-20. http://dx.doi.org/10.1358/dot.2015.51.1.2250387 PMID: 25685857

[14] Lee HT, Lee JY, Lim H, *et al.* Molecular mechanism of PD-1/PD-L1 blockade *via* anti-PD-L1 antibodies atezolizumab and durvalumab. Sci Rep 2017; 7(1): 5532. http://dx.doi.org/10.1038/s41598-017-06002-8 PMID: 28717238

[15] Buchbinder Elizabeth I. Desai Anupam. CTLA-4 and PD-1 Pathways: Similarities, differences, and implications of their inhibition. Am J Clin Oncol 2016. http://dx.doi.org/10.1097/COC.0000000000000239

[16] Chae YK, Arya A, Iams W, *et al.* Current landscape and future of dual anti-CTLA4 and PD-1/PD-L1 blockade immunotherapy in cancer; lessons learned from clinical trials with melanoma and Non-Small Cell Lung Cancer (NSCLC). J Immunother Cancer 2018; 6(1): 39. http://dx.doi.org/10.1186/s40425-018-0349-3 PMID: 29769148

[17] Engelhardt JJ, Sullivan TJ, Allison JP. CTLA-4 overexpression inhibits T cell responses through a CD28-B7-dependent mechanism. J Immunol 2006; 177(2): 1052-61. http://dx.doi.org/10.4049/jimmunol.177.2.1052 PMID: 16818761

[18] Paulsen EE, Kilvaer TK, Rakaee M, *et al.* CTLA-4 expression in the non-small cell lung cancer patient tumor microenvironment: Diverging prognostic impact in primary tumors and lymph node metastases. Cancer Immunol Immunother 2017; 66(11): 1449-61. http://dx.doi.org/10.1007/s00262-017-2039-2 PMID: 28707078

[19] Granier C, De Guillebon E, Blanc C, *et al.* Mechanisms of action and rationale for the use of checkpoint inhibitors in cancer. ESMO Open 2017; 2(2)e000213 http://dx.doi.org/10.1136/esmoopen-2017-000213 PMID: 28761757

[20] Grosso JF, Jure-Kunkel MN. CTLA-4 blockade in tumor models: An overview of preclinical and translational research. Cancer Immun 2013; 13: 5. PMID: 23390376

[21] He Y, Yu H, Rozeboom L, *et al.* LAG-3 protein expression in non-small cell lung cancer and its relationship with PD-1/PD-L1 and tumor-infiltrating lymphocytes. J Thorac Oncol 2017; 12(5): 814-23. http://dx.doi.org/10.1016/j.jtho.2017.01.019 PMID: 28132868

[22] Hald SM, Rakaee M, Martinez I, *et al.* LAG-3 in Non-small-cell lung cancer: Expression in primary tumors and metastatic lymph nodes is associated with improved survival. Clin Lung Cancer 2018; 19(3): 249-259. http://dx.doi.org/10.1016/j.cllc.2017.12.001 PMID: 29396238

[23] Goldberg MV, Drake CG. LAG-3 in Cancer immunotherapy. Curr Top Microbiol Immunol 2011; 344: 269-78. http://dx.doi.org/10.1007/82_2010_114 PMID: 21086108

[24] Das M, Zhu C, Kuchroo VK. Tim-3 and its role in regulating anti-tumor immunity. Immunol Rev 2017; 276(1): 97-111. http://dx.doi.org/10.1111/imr.12520 PMID: 28258697

[25] Du W, Yang M, Turner A, *et al.* TIM-3 as a Target for cancer immunotherapy and mechanisms of action. Int J Mol Sci 2017; 18(3): 645. http://dx.doi.org/10.3390/ijms18030645 PMID: 28300768

[26] Leitner J, Klauser C, Pickl WF, *et al.* B7-H3 is a potent inhibitor of human T-cell activation: No evidence for B7-H3 and TREML2 interaction. Eur J Immunol 2009; 39(7): 1754-64. http://dx.doi.org/10.1002/eji.200839028 PMID: 19544488

[27] Castellanos JR, Purvis IJ, Labak CM, *et al.* B7-H3 role in the immune landscape of cancer. Am J Clin Exp Immunol 2017; 6(4): 66-75. PMID: 28695059

[28] Altan M, Pelekanou V, Schalper KA, *et al.* B7-H3 Expression in NSCLC and its association with B7-H4, PD-L1 and tumor-infiltrating lymphocytes. Clin Cancer Res 2017; 23(17): 5202-9. http://dx.doi.org/10.1158/1078-0432.CCR-16-3107 PMID: 28539467

[29] Lee YH, Martin-Orozco N, Zheng P, *et al.* Inhibition of the B7-H3 immune checkpoint limits tumor growth by enhancing cytotoxic lymphocyte function. Cell Res 2017; 27(8): 1034-45. http://dx.doi.org/10.1038/cr.2017.90 PMID: 28685773

[30] Picarda E, Ohaegbulam KC, Zang X. Molecular pathways: Targeting B7-H3 (CD276) for human cancer immunotherapy. Clin Cancer Res 2016; 22(14): 3425-31.

http://dx.doi.org/10.1158/1078-0432.CCR-15-2428 PMID: 27208063

[31] Lines JL, Sempere LF, Broughton T, Wang L, Noelle R. VISTA is a novel broad-spectrum negative checkpoint regulator for cancer immunotherapy. Cancer Immunol Res 2014; 2(6): 510-7. http://dx.doi.org/10.1158/2326-6066.CIR-14-0072 PMID: 24894088

[32] Villarroel-Espindola F, Yu X, Datar I, *et al.* Spatially resolved and quantitative analysis of VISTA/PD-1H as a novel immunotherapy target in human non-small cell lung cancer. Clin Cancer Res 2018; 24(7): 1562-73. http://dx.doi.org/10.1158/1078-0432.CCR-17-2542 PMID: 29203588

[33] Dempke WCM, Fenchel K, Uciechowski P, Dale SP. Second- and third-generation drugs for immuno-oncology treatment-The more the better? Eur J Cancer 2017; 74: 55-72. http://dx.doi.org/10.1016/j.ejca.2017.01.001 PMID: 28335888

[34] Campbell KS, Purdy AK. Structure/function of human killer cell immunoglobulin-like receptors: Lessons from polymorphisms, evolution, crystal structures and mutations. Immunology 2011; 132(3): 315-25. http://dx.doi.org/10.1111/j.1365-2567.2010.03398.x PMID: 21214544

[35] Al Omar SY, Marshall E, Middleton D, Christmas SE. Increased killer immunoglobulin-like receptor expression and functional defects in natural killer cells in lung cancer. Immunology 2011; 133(1): 94-104. http://dx.doi.org/10.1111/j.1365-2567.2011.03415.x PMID: 21342183

[36] Benson DM Jr, Caligiuri MA. Killer immunoglobulin-like receptors and tumor immunity. Cancer Immunol Res 2014; 2(2): 99-104. http://dx.doi.org/10.1158/2326-6066.CIR-13-0219 PMID: 24592397

[37] He Y, Liu S, Mattei J, Bunn PA Jr, Zhou C, Chan D. The combination of anti-KIR monoclonal antibodies with anti-PD-1/PD-L1 monoclonal antibodies could be a critical breakthrough in overcoming tumor immune escape in NSCLC. Drug Des Devel Ther 2018; 12: 981-6. http://dx.doi.org/10.2147/DDDT.S163304 PMID: 29731605

[38] Vinay DS, Kwon BS. 4-1BB (CD137), an inducible co-stimulatory receptor, as a specific target for cancer therapy. BMB Rep 2014; 47(3): 122-9. http://dx.doi.org/10.5483/BMBRep.2014.47.3.283 PMID: 24499671

[39] Wang C, Lin GH, McPherson AJ, Watts TH. Immune regulation by 4-1BB and 4-1BBL: Complexities and challenges. Immunol Rev 2009; 229(1): 192-215. http://dx.doi.org/10.1111/j.1600-065X.2009.00765.x PMID: 19426223

[40] Chester C, Sanmamed MF, Wang J, Melero I. Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies. Blood 2018; 131(1): 49-57. http://dx.doi.org/10.1182/blood-2017-06-741041 PMID: 29118009

[41] Chester C, Ambulkar S, Kohrt HE. 4-1BB agonism: Adding the accelerator to cancer immunotherapy. Cancer Immunol Immunother 2016; 65(10): 1243-8. http://dx.doi.org/10.1007/s00262-016-1829-2 PMID: 27034234

[42] Croft M, So T, Duan W, Soroosh P. The significance of OX40 and OX40L to T-cell biology and immune disease. Immunol Rev 2009; 229(1): 173-91. http://dx.doi.org/10.1111/j.1600-065X.2009.00766.x PMID: 19426222

[43] Jensen SM, Maston LD, Gough MJ, *et al.* Signaling through OX40 enhances antitumor immunity. Semin Oncol 2010; 37(5): 524-32. http://dx.doi.org/10.1053/j.seminoncol.2010.09.013 PMID: 21074068

[44] Carrizosa DR, Gold KA. New strategies in immunotherapy for non-small cell lung cancer. Transl Lung Cancer Res 2015; 4(5): 553-9. PMID: 26629424

[45] Nocentini G, Ronchetti S, Cuzzocrea S, Riccardi C. GITR/GITRL: More than an effector T cell co-stimulatory system. Eur J Immunol 2007; 37(5): 1165-9. http://dx.doi.org/10.1002/eji.200636933 PMID: 17407102

[46] Riether C, Schürch C, Ochsenbein AF. Modulating CD27 signaling to treat cancer. OncoImmunology 2012; 1(9): 1604-6. http://dx.doi.org/10.4161/onci.21425 PMID: 23264908

[47] Knee DA, Hewes B, Brogdon JL. Rationale for anti-GITR cancer immunotherapy. Eur J Cancer 2016; 67: 1-10. http://dx.doi.org/10.1016/j.ejca.2016.06.028 PMID: 27591414

[48] van de Ven K, Borst J. Targeting the T-cell co-stimulatory CD27/CD70 pathway in cancer immunotherapy: Rationale and potential. Immunotherapy 2015; 7(6): 655-67. http://dx.doi.org/10.2217/imt.15.32 PMID: 26098609

[49] Hornyák L, Dobos N, Koncz G, *et al.* The Role of indoleamine-2, 3-dioxygenase in cancer development, diagnostics, and therapy. Front Immunol 2018; 9: 151. http://dx.doi.org/10.3389/fimmu.2018.00151 PMID: 29445380

[50] Zhai L, Ladomersky E, Lenzen A, *et al.* IDO1 in cancer: A gemini of immune checkpoints. Cell Mol Immunol 2018; 15(5): 447-57. http://dx.doi.org/10.1038/cmi.2017.143 PMID: 29375124

[51] Volaric A, Gentzler R, Hall R, *et al.* Indoleamine-2,3-dioxygenase in non-small cell lung cancer: A targetable mechanism of immune resistance frequently coexpressed with PD-L1. Am J Surg Pathol 2018; 42(9): 1216-23. http://dx.doi.org/10.1097/PAS.0000000000001099 PMID: 29901571

[52] Schalper KA, Carvajal-Hausdorf D, McLaughlin J, *et al.* Differential expression and significance of PD-L1, IDO-1, and B7-H4 in human lung cancer. Clin Cancer Res 2017; 23(2): 370-8. http://dx.doi.org/10.1158/1078-0432.CCR-16-0150 PMID: 27440266

[53] Li F, Zhang R, Li S, Liu J. IDO1: An important immunotherapy target in cancer treatment. Int Immunopharmacol 2017; 47: 70-7. http://dx.doi.org/10.1016/j.intimp.2017.03.024 PMID: 28365507

[54] Liu M, Wang X, Wang L, *et al.* Targeting the IDO1 pathway in cancer: From bench to bedside. J Hematol Oncol 2018; 11(1): 100. http://dx.doi.org/10.1186/s13045-018-0644-y PMID: 30068361

[55] Brochez L, Chevolet I, Kruse V. The rationale of indoleamine 2,3-dioxygenase inhibition for cancer therapy. Eur J Cancer 2017; 76: 167-82. http://dx.doi.org/10.1016/j.ejca.2017.01.011 PMID: 28324751

[56] Fishman P, Bar-Yehuda S, Synowitz M, *et al.* Adenosine receptors and cancer. Handb Exp Pharmacol 2009; (193): 399-441. http://dx.doi.org/10.1007/978-3-540-89615-9_14 PMID: 19639290

[57] Leone RD, Lo YC, Powell JD. 2015.

[58] Leone RD, Emens LA. Targeting adenosine for cancer immunotherapy. J Immunother Cancer 2018; 6(1): 57. http://dx.doi.org/10.1186/s40425-018-0360-8 PMID: 29914571

[59] Massagué J. TGFbeta in cancer. Cell 2008; 134(2): 215-30. http://dx.doi.org/10.1016/j.cell.2008.07.001 PMID: 18662538

[60] Jakowlew SB. Transforming growth factor-beta in cancer and metastasis. Cancer Metastasis Rev 2006; 25(3): 435-57. http://dx.doi.org/10.1007/s10555-006-9006-2 PMID: 16951986

[61] Colak S, Ten Dijke P. Targeting TGF-β signaling in cancer. Trends Cancer 2017; 3(1): 56-71. http://dx.doi.org/10.1016/j.trecan.2016.11.008 PMID: 28718426

[62] de Gramont A, Faivre S, Raymond E. Novel TGF-β inhibitors ready for prime time in onco-immunology. OncoImmunology 2016; 6(1)e1257453 http://dx.doi.org/10.1080/2162402X.2016.1257453 PMID: 28197376

[63] Katz LH, Li Y, Chen JS, *et al.* Targeting TGF-β signaling in cancer. Expert Opin Ther Targets 2013; 17(7): 743-60. http://dx.doi.org/10.1517/14728222.2013.782287 PMID: 23651053

[64] Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell 2017; 168(4): 707-23. http://dx.doi.org/10.1016/j.cell.2017.01.017 PMID: 28187290

[65] Rieth J, Subramanian S. Mechanisms of intrinsic tumor resistance to immunotherapy. Int J Mol Sci 2018; 19(5): 1340. http://dx.doi.org/10.3390/ijms19051340 PMID: 29724044

[66] Herbst RS, Baas P, Kim DW, *et al.* Pembrolizumab *vs.* docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): A randomised controlled trial. Lancet 2015. PMID: 26712084

[67] Borghaei H, Paz-Ares L, Horn L, *et al.* Nivolumab *vs.* docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015; 373(17): 1627-39. http://dx.doi.org/10.1056/NEJMoa1507643 PMID: 26412456

[68] Brahmer J, Reckamp KL, Baas P, *et al.* Nivolumab *vs.* docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015; 373(2): 123-35. http://dx.doi.org/10.1056/NEJMoa1504627 PMID: 26028407

[69] Rittmeyer A, Barlesi F, Waterkamp D, *et al.* Atezolizumab *vs.* docetaxel in patients with previously treated non-small-cell lung cancer (OAK): A phase 3, open-label, multicentre randomised controlled trial. Lancet 2016. PMID: 27979383

[70] Schmidt C. The benefits of immunotherapy combinations. Nature 2017; 552(7685): S67-9. http://dx.doi.org/10.1038/d41586-017-08702-7 PMID: 29293245

[71] Harris SJ, Brown J, Lopez J, Yap TA. Immuno-oncology combinations: Raising the tail of the survival curve. Cancer Biol Med 2016; 13(2): 171-93. http://dx.doi.org/10.20892/j.issn.2095-3941.2016.0015 PMID: 27458526

[72] Ott PA, Hodi FS, Kaufman HL, Wigginton JM, Wolchok JD. Combination immunotherapy: A road map. J Immunother Cancer 2017; 5: 16. http://dx.doi.org/10.1186/s40425-017-0218-5 PMID: 28239469

[73] Antonia S, Goldberg SB, Balmanoukian A, *et al.* Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: A multicentre, phase 1b study. Lancet Oncol 2016; 17(3): 299-308. http://dx.doi.org/10.1016/S1470-2045(15)00544-6 PMID: 26858122

[74] Mok T, Schmid P, Arén O, *et al.* 192TiP: NEPTUNE: A global, phase 3 study of durvalumab (MEDI4736) plus tremelimumab combination therapy *vs.* Standard of Care (SoC) platinum-based chemotherapy in the first-line treatment of patients (pts) with advanced or metastatic NSCLC. J Thorac Oncol 2016; 11(4): 140-1. http://dx.doi.org/10.1016/S1556-0864(16)30301-X

[75] Rizvi N, Barlesi F, Brahmer J, *et al.* Phase III, randomized, open-label study of durvalumab (MEDI4736) in combination with tremelimumab or durvalumab alone *vs.* platinum-based chemotherapy in first-line treatment of patients with advanced/metastatic NSCLC: MYSTIC. J Immunother Cancer. 2015; 3(Suppl 2): P171. http://dx.doi.org/10.1186/2051-1426-3-S2-P171

[76] Planchard D, Yokoi T, McCleod MJ, *et al.* A Phase III study of durvalumab (MEDI4736) with or without tremelimumab for previously treated patients with advanced NSCLC: rationale and protocol design of the ARCTIC study. Clin Lung Cancer 2016; 17(3): 232-236.e1. http://dx.doi.org/10.1016/j.cllc.2016.03.003 PMID: 27265743

[77] Muñoz-Unceta N, Burgueño I, Jiménez E, Paz-Ares L. Durvalumab in NSCLC: latest evidence and clinical potential. Ther Adv Med Oncol 2018; 101758835918804151 http://dx.doi.org/10.1177/1758835918804151 PMID: 30344651

[78] Gubens MA, Sequist LV, Stevenson J, *et al.* Phase I/II study of pembrolizumab (pembro) plus ipilimumab (ipi) as second-line therapy for NSCLC: KEYNOTE-021 cohorts D and H. J Clin Oncol 2016; 34. http://dx.doi.org/10.1200/JCO.2016.34.15_suppl.9027

[79] ClinicalTrials.gov [Internet]. Bethesda (MD): National Li-brary of Medicine (US) Study of pembrolizumab given with ipilimumab or placebo in participants with untreated meta-static non-small cell lung cancer (MK-3475-598/KEYNOTE-598); identifier: NCT03302234; available at: https://clinicaltrials.gov/ct2/show/NCT03302234 [Accessed December 2018].

[80] Hellmann MD, Rizvi NA, Goldman JW, *et al.* Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. Lancet Oncol 2017; 18(1): 31-41. http://dx.doi.org/10.1016/S1470-2045(16)30624-6 PMID: 27932067

[81] Ramalingam SS, Hellmann MD, Awad MM, *et al*. Tumor mutational burden (TMB) as a biomarker for clinical benefit from dual immune checkpoint blockade with nivolumab (nivo) + ipilimumab (ipi) in first-line (1L) Non-Small Cell Lung Cancer (NSCLC): Identification of TMB cutoff from Check Mate 568. Cancer Res 2018; 78: CT078. http://dx.doi.org/10.1158/1538-7445.AM2018-CT078

[82] Hellmann MD, Ciuleanu T-E, Pluzanski A, *et al*. Nivolumab plus Ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med 2018; 378(22): 2093-104.

[83] BMS Press Release [internet] Bristol-Myers Squibb Provides Update on the Ongoing Regulatory Review of Opdivo Plus Low-Dose Yervoy in First-Line Lung Cancer Patients with Tumor Mutational Burden ≥10 mut/Mb. Available at: https://news.bms.com/press-release/corporatefinancial-news/bristol-myers-squibb-provides-update-ongoing-regulatory-review [Accessed December 2018].