Immunotherapy in non-small-cell lung cancer: from targeted molecules to resistance patterns

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Short running title

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Author Name

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Abstract

Immunotherapies are now considered as a pillar of non-small cell lung cancer (NSCLC) treatment. The main targets of immune-checkpoint inhibitors (ICI) are programmed cell death 1 (PD-1) / programmed cell death ligand 1 (PD-L1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4), aiming at restoring antitumor immunity. Despite durable responses observed in some patients, all the

patients do not benefit from the treatment and almost all responders ultimately relapse after some time. In this review, we discuss the biomarkers that could be used to predict response to ICI, the current indications of ICI in NSCLC, the mechanisms inducing tumor-cell intrinsic or extrinsic resistance to ICI and finally, the potential treatment response monitoring.

Keywords

Immunotherapy, immune checkpoint inhibitor, PD-L1, mechanisms of resistance, molecular profile

Main body of text

I- Introduction

Lung cancer remains a leading cause of cancer death worldwide with more than a million deaths per year. Treatment of non-small cell lung carcinoma (NSCLC) mainly depends on the presence of somatic oncogenic drivers. Tumors harboring *EGFR / BRAF* activating mutations or *ALK / ROS* rearrangement may benefit from targeted therapy, which significantly prolongs survival. In contrast, therapeutic approaches for advanced NSCLC without oncogenic drivers have been limited for a long time to chemotherapy. In the past decade, cumulative evidence supporting the key role of the immune system in NCSLC development in conjunction with genomic and molecular pathways analyses has led to the development of immunotherapies (IT). IT are now considered as a pillar of NSCLC treatment. Different molecular targets have been reported (Table I). Among them, the immune checkpoint receptor cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) negatively regulates T-cell activation in NSCLC and represents a target of choice in IT. Programmed death-ligand 1 (PD-L1) also plays a key role in immune-regulation. It inhibits the $CD8^+$ cytotoxic immune response through its receptor (PD-1) binding. PD-L1 could be over-expressed in tumor cells, such as lung cancer cells. Targeted therapies aiming at blocking the PD-L1/PD-1 signaling pathway are now commonly used in daily practice for NSCLC. However, despite the important potential impact on survival, only a small proportion of patients benefit from IT. Moreover, different intrinsic and/or extrinsic mechanisms that confer resistance to IT have been identified in the last few years. In this review, we will focus on the different predictive biomarkers of response to IT, summarize the current indications of ICI in NSCLC and describe the mechanisms of IT resistance.

II - Update on predictive biomarkers for ICI and emerging novel targets

The most well-known and validated immune checkpoints are CTLA-4 and PD-1, which are negative regulators of T-cells in distinct immune microenvironments. They prevent autoimmunity and limit immune activation. They are non-redundant in the co-inhibitory pathways [1,2]. Comparisons showed that PD-1 engagement interferes with more T-cell signaling pathways than CTLA-4 engagement does [3]. T-cells are activated by the interaction between B7 ligands of antigen-presenting cells (APC) and CD28 on T-cells. CTLA-4 outcompetes CD28 with a competitive and stronger binding to B7, and then suppresses T-cell activation. Cancer Immune Checkpoint Inhibitors (ICI) using anti-CTLA-4 antibodies block this CTLA-4/B7 interaction. T-cell activation is also suppressed by the interaction between PD-1 and PD-L1. PD-1 is homologous to CD28 and has two ligands: PD-L1 and PD-L2, both detected on antigen-presenting cells, but otherwise expressed by different somatic cells [2,5]. Cancer cells and tumor-infiltrating immune cells (such as macrophages) also express PD-L1. Tumor cells frequently express high PD-L1 levels to decrease T-cell activation. ICI block the PD-1/PD-L1 interaction and promote anti-tumor activity [1,4]. IFN-γ can regulate PD-L1 expression and different *JAK-3* mutations also increase PD-L1 expression in NSCLC [5–7].

Despite the high impact of ICI, variable clinical outcomes have been described. Many patients are non-responders in daily practice. In NSCLC, PD-L1 expression is the only approved predictive marker for PD-1/PD-L1 blockade. PD-L1 expression is evaluated using immunohistochemistry. Nevertheless, anti-PD-L1 or PD-1 ICI will mainly benefit patients harboring high PD-L1 expression tumors [7]. Additional cancer biomarkers, as predictors of IT response, are essential to distinguish responders from non-responders before IT treatment [8]

In NSCLC, anti-CTLA-4 and -PD-L1 ICI are also described to be more effective in tumors harboring high TMB (Tumor Mutational Burden) or MSI (MicroSatellite Instability) phenotype [5]**.** TMB is the number of non-synonymous somatic mutations in the coding genome. Frameshift or indel mutations have been described to be more immunogenic than missense mutations. TMB is associated with smoking and *TP53* mutations. TMB is negatively associated with *EGFR* mutations and clinical outcomes in patients with *EGFR*-mutant advanced lung cancer treated with EGFR Tyrosine Kinase Inhibitors (TKI). More recently, a significant association between somatic

RYR2 (ryanodine receptor 2) mutations and high TMB was defined as a potential prognostic marker for lung adenocarcinoma [9]. Other authors have developed a 25 miRNA-based signature classifier, involved in immune-related biological processes and in cancer-related pathways, as a potential biomarker to predict TMB levels in lung adenocarcinoma [10]. High TMB can increase neo-antigen expression linked to T-cell recruitment [4]. A higher migratory capacity of $CD8^+$ T-cells has been described in patients classified as responders before IT [12]. Correlations have been found between high TMB and increased tumor-infiltrating lymphocytes (TILs), expression of cytokines and immune-related genes, though the impact on clinical outcome needs to be better understood [7]. Moreover, TMB is described as a potential predictor of IT response for lung tumors [11], but TMB alone seems insufficient to predict response to ICI in NSCLC [7]. Willis's recent meta-analysis of patients receiving IT for lung cancer reported that a high TMB (≥ 10 mut/Mb) was associated with greater benefits, notably ORR (Overall Response Rate) and PFS (Progression Free Survival). Nevertheless, the association between PD-L1 expression and TMB is inconsistent and appear as independent factors of ICI response [11]. At this time, WES (Whole Exome Sequencing) remains the gold standard for the evaluation of TMB. However, WES is not available in all laboratories at this time. The use of large comprehensive panels (more than 350 genes) appears to be a good alternative to WES for TMB evaluation. The exact threshold that defines high TMB remains unclear at this time. This lack of standardization limits the comparison between the different clinical trials and the use of TMB in daily practice.

High Microsatellite instability (MSI-H) and dMMR (DNA mismatch repair deficiency) are hallmarks of tumor genome instability. These tumors are frequently associated with increased TMB [8]. However, many high TMB tumors do not exhibit dMMR/MSI-H phenotype and may still benefit from IT. Willis *et al.* suggested that dMMR/MSI-H status used in conjunction with PD-L1 expression level and TMB evaluation may be used to identify IT responders. In 2017, the US FDA approved pembrolizumab for use in adult and pediatric patients with unresectable or metastatic solid tumors with positive MSI-H or dMMR biomarkers. This was first "tissue-agnostic" biomarker to receive FDA approval though additional trials are needed and experimental verification is ongoing [4,8,11].

Other clinical challenges to address include the impact of tumor microenvironment on tumor evolution. Tumor microenvironment modulates immune and stromal cell functions, and influence

tumor heterogeneity [13]. For example, increased chemokine synthesis (such as IFN-γ and CXCL9) could be evaluated before treatment as micro-environmental immunity biomarkers [6]. IFN-γ released by infiltrating immune cells (T cells, NK cells) can upregulate PD-L1 expression and seems to be associated with better prognosis [5–7]. In NSCLC patients treated with nivolumab, high IFN-γ mRNA expression was associated with improved median PFS [7]. However, *JAK1*/*2* mutations limit the positive effect of IFN-γ [2]. Both PD-1 and CTLA-4 blockades are more effective in tumors infiltrated by T-cells. In melanoma and NSCLC, the efficacy of pembrolizumab is improved by increased tumor T-Cell immune infiltrate [4]. In a meta-analysis of NSCLC, Zeng *et al.* [7] reported that $CD8^+$ T-cell infiltrate was the best predictor of survival. Future validation of TIL composition in NSCLC could help to develop treatment strategies, (*e.g.* the stromal expression analysis of PD-L1, $CD3^+$, $CD4^+$ and $CD8^+$) [7]. In melanoma, Krieg *et al* found that the frequency of classical monocytes with up-regulation of PD-L1, may predict responsiveness to anti-PD-1 IT [12]. Peranzoni *et al.* on the other hand suggested improving cancer IT efficacy by targeting macrophages, which impedes T cells and limits the efficacy of anti-PD-1 treatment [14]. Another approach uses the levels of tumor microenvironment inflammation, for example the tumor inflammation signature (TIS), to predict the clinical benefit of IT. TIS scores for the 18-gene tumor inflammation signature were significantly associated with complete or partial response to anti-PD1 treatment in a cohort including NSCLC patients [15]. The role of myeloid cells in inflammation and cancer is the source of ongoing debate. Krieg *et al.* described that in melanoma, the increased number of tumor-infiltrating lymphocytes in responders is indicative of an increase in myeloid cell frequency when T cells move from the blood to the tumor.

In regard to host-related biomarkers, the microbiome in the gut may modulate and improve ICI therapies [5,16], but more experimental settings are needed. High serum LDH (Lactate Dehydrogenase) levels have been detected in cancer cells consuming high levels of glucose and are associated with poor prognosis in solid tumors including lung tumors. Larger studies are needed to assess the prognostic impact of elevated LDH on NSCLC treated with ICI [7].

The need for alternative treatments to replace PD-1/PD-L1 and CTLA-4 blockades and reduce the risk of patient relapse of patients has led to the identification of alternative inhibitory receptors: LAG-3 (Lymphocyte activation gene-3), TIGIT (T cell immunoglobulin and ITIM domain), TIM3 (T cell immunoglobulin-3) [17,18], VISTA (V-domain Ig-containing suppressor of T-cell activation) [19] and many others are currently under investigation. Of particular interest is LAG-

3, an inhibitor of immune response that prevents autoimmunity and is the third inhibitory receptor to be targeted in clinical trials [18]. A promising synergy has been found between LAG-3 and PD1 blockade and different clinical trials are exploring LAG3-based immune checkpoint blockades [18]. TIM-3 positive tumor-infiltrating lymphocytes co-express PD-1, suggesting a potential synergistic effect between these two checkpoint co-inhibitors [20]. VISTA, an immune checkpoint gene that inhibits anti-tumor immune responses, was studied in malignant pleural mesothelioma, which displays the highest expression of VISTA among all cancers studied [19]. Muller *et al.* reported frequent expression of VISTA and infrequent expression of PD-L1 with favorable and unfavorable survival correlations, respectively. These findings may explain the poor responses to anti-PD-L1, and suggest VISTA as a potential novel target in pleural mesothelioma. New studies are pending.

Along with tumor histogenesis classification, biomarker-based disease classification is now a requirement in the design of new therapies, notably to better target immune checkpoints. Sequential or combination blockades are currently under investigation. Recent structural studies have provided explanations about molecular interactions of ICI and will improve the design of combination IT with synergistic antitumor effects [1].

III - Current indications of ICI in NSCLC

Accumulative evidence has revealed that immune evasion is one of the hallmarks of malignant cells to escape antitumor immune responses. Therefore, ICI now plays a key role in the treatment of advanced NSCLC, both in first and second lines of therapy. Hence, different clinical trials have evaluated anti-PD-1, -PD-L1 and/or -CTLA-4 antibodies as a single therapeutic agent or in combination with chemotherapy and/or another ICI in advanced NSCLC. In this section, we will only focus on the main clinical trials which have led to FDA (Food and Drug Administration) or EMA (European Medicines Agency) approval of ICI for the treatment of non-squamous NSCLC (Table II).

1- ICI monotherapy in the first-line treatment in advanced NSCLC

KEYNOTE-024 clinical trial compared pembrolizumab vs platinum-based chemotherapy in first-line treatment of patients with PD-L1 ≥50% advanced NSCLC. Median PFS and OS (Overall Survival) were significantly longer with pembrolizumab vs chemotherapy (10.3 vs 6.0 months; HR

0.50; p<0.001 and 30.0 vs 14.2 months; HR 0.63; p=0.002, respectively), while the RR was also higher (44.8% vs 27.8%, respectively). Moreover, 54% of patients initially treated by chemotherapy were crossed over to receive pembrolizumab [21,22]. FDA and EMA approved pembrolizumab in first-line treatment of PD-L1 ≥50% advanced NSCLC.

KEYNOTE-042 phase 3 clinical trial enrolled patients with PD-L1 expression ≥1% advanced NSCLC to compare pembrolizumab vs platinum-based chemotherapy in first-line treatment. In contrast to KEYNOTE-024, PFS was not significantly improved with pembrolizumab treatment, even in PD-L1 ≥50% tumors (7.1 vs 6.4 months; HR 0.81; p=0.0170). Nonetheless, OS were significantly longer with pembrolizumab vs chemotherapy in PD-L1 ≥1% population (16.7 vs 12.1 months; HR 0.81; p=0.0018). Interestingly, OS seemed to be similar in the PD-L1 expression of the 1–49% subgroup (13.4 vs 12.1 months; HR 0.92) [23].These results however gave rise to the FDA approval of pembrolizumab for the first-line treatment of patients with stage III or metastatic NSCLC and tumor PD-L1 expression ≥1%. Tumors harboring *EGFR* activating mutations or *ALK* rearrangement must first be treated by specific tyrosine kinase inhibitors before receiving ICI. Further investigations are needed to explore whether pembrolizumab may be useful in PD-L1 ≥1% population.

Unlike pembrolizumab, neither nivolumab nor atezolizumab monotherapy have been approved yet in first line treatment of NSCLC. CheckMate 026 phase III trial compared nivolumab vs chemotherapy in first line treatment of patients with PD-L1 ≥5% tumors. Median PFS and OS were not significantly longer in the nivolumab group (4.2 vs 5.9 months; HR 1.15; p=0.25 and 14.4 vs 13.2 months; HR 1.02, respectively) [24]. Likewise, IMpower110 included 554 patients to compare atezolizumab vs chemotherapy in first line treatment of PD-L1 positive stage IV NSCLC. Median OS was not significantly longer with atezolizumab (17.5 vs 14.1 months; HR 0.83; p=0.15) [25].

2- Combination of ICI and chemotherapy or ICI in the first-line treatment in advanced NSCLC

Pembrolizumab

Despite an important benefit both in PFS and OS, the ORR of pembrolizumab monotherapy in first line treatment was only 44% in KEYNOTE-024 clinical trial [21]. To improve this ORR, ICI were assessed in combination with chemotherapy. KEYNOTE-189 phase 3 trial compared platinum-based therapy and pemetrexed with or without pembrolizumab in previously untreated advanced

NSCLC. Estimated OS rate at 12 months was 69.2% in the chemotherapy-pembrolizumab-combination group vs 49.4% in the chemotherapy group (HR 0.49; p<0.001). The OS benefit was observed across all PD-L1 expression subgroups, even in those with <1% PD-L1 expression (12-month OS rate 61.7% vs 52.2%; HR 0.59). As expected, an increased expression of PD-L1 improved OS rate. Median PFS was significantly longer with the addition of pembrolizumab compared with the chemotherapy group (8.8 vs 4.9 months; HR 0.52; p<0.001) [26]. FDA and EMA approved pembrolizumab in combination with chemotherapy for first-line treatment of metastatic non-squamous NSCLC. After a 18.7-month median follow up, updated analysis showed longer PFS and OS with pembrolizumab plus chemotherapy irrespective of PD-L1 tumor expression (9.0 vs 4.9 months; HR 0.48 and 22.0 vs 10.7 months; HR 0.56; p<0.00001, respectively) [27].

Nivolumab

CheckMate 227 is a multipart phase 3 trial which enrolled previously untreated patients with advanced NSCLC. TMB was evaluated by FoundationOne CDx assay and defined as the number of somatic mutations per megabase of sequenced genome (high TMB: ≥10 mutations per megabase). Among patients with high TMB, the 1-year PFS rate and ORR were significantly higher with nivolumab plus ipilimumab vs chemotherapy (42.6% vs 13.2% and 45.3% vs 26.9%, respectively). Median PFS was also longer (7.2 vs 5.5 months; HR 0.58; p<0.001) [28]. Regardless of the PD-L1 expression level, median OS was also longer with nivolumab plus ipilimumab vs chemotherapy (17.1 vs 13.9 months; HR 0.73). The OS benefit was observed in patients with a PD-L1 expression ≥1% or <1% subgroups (17.1 vs 14.9 months; HR 0.79; p=0.007 and 17.2 vs 12.2 months; HR 0.62, respectively) [29]. Based on these results, a FDA priority review was granted to the combination nivolumab plus ipilimumab in first line treatment of advanced NSCLC.

Different studies recently included TMB as a biomarker of predictive response to ICI. For example, the B-F1RST phase II study included patients with untreated advanced NSCLC to receive first line atezolizumab until progression. The ORR, PFS and OS were higher in patients with high blood based TMB (≥16 Mutation/Mb) vs low TMB (28.6 vs 4.4%; 5.0 vs 3.5 months; HR 0.80 and 23.9 vs 13.4 months; HR 0.66, respectively) [30].

Atezolizumab

IMpower150 multipart clinical trial compared atezolizumab in combination with chemotherapy (carboplatin and paclitaxel), with (ABCP) or without (ACP) bevacizumab vs bevacizumab plus

chemotherapy (BCP) in first line treatment of stage IV non-squamous NSCLC. The addition of atezolizumab to BCP significantly improved the median PFS and OS (8.3 months vs 6.8 months; HR 0.62; p<0.001 and 19.2 vs 14.7 months; HR 0.78; p=0.02, respectively). Based on these results, FDA and EMA approved the association of ABCP in first line treatment of metastatic non-squamous NSCLC not harboring *EGFR* or *ALK* genomic tumor alterations. Moreover, among patients harboring tumors with *EGFR* or *ALK* genomic tumor alterations (n=108), median PFS and OS were also significantly improved in the ABCP group compared with those in the BCP group (9.7 vs 6.1 months, HR 0.59 and not reached vs 17.5 months, HR 0.54, respectively) [31] [32]. In 2019, EMA approved ABCP for these patients after failure of appropriate targeted therapies.

In the same way, IMpower130 study compared carboplatin and nab-paclitaxel with or without atezolizumab in first line treatment of stage IV non-squamous NSCLC. Median PFS and OS were significantly longer in the atezolizumab plus chemotherapy group vs chemotherapy alone (7.0 vs 5.5 months; HR 0.64; p<0.0001 and 18.6 vs 13.9 months; HR 0.79; p=0.033, respectively) [33]. FDA recently approved atezolizumab plus chemotherapy (carboplatin and nab-paclitaxel) in first line treatment of NSCLC in patients with no *EGFR* or *ALK* genomic tumor aberrations.

3- ICI in previously treated advanced NSCLC

Pembrolizumab

One of the first approvals of ICI in NSCLC was pembrolizumab in 2015. KEYNOTE-010 phase III clinical trial compared pembrolizumab vs docetaxel in previously treated PD-L1 positive advanced NSCLC. In the overall population, there was no significant difference in PFS between the two groups. In contrast, OS was significantly longer with pembrolizumab (12.7 vs 8.5 months; HR 0.61; p<0.0001). In the PD-L1 ≥50% tumor subgroup, median PFS and OS were significantly longer with pembrolizumab (5.2 vs 4.1 months; HR 0.59; p<0.0001 and 17.3 vs 8.2 months; HR 0.50; p<0.0001, respectively) [34]. Based on these findings, pembrolizumab was approved as a monotherapy for the treatment of patients with previously treated metastatic PD-L1 positive (≥1%) NSCLC. After a follow up of 43 months, the updated results were consistent with the first published conclusions. OS was significantly longer with pembrolizumab (11.8 vs 8.4 months; HR 0.69; p<0.00001 and 16.9 vs 8.2 months; HR 0.53; p<0.00001, respectively) in both the overall population and PD-L1 ≥50% tumor subgroup [35].

Nivolumab

Similarly, CheckMate 57 phase III clinical trial enrolled patients with non-squamous NSCLC that had progressed after chemotherapy to receive nivolumab or docetaxel. Despite a moderate response rate of 19% with nivolumab vs 12% with docetaxel (p=0.02), median OS was longer with nivolumab vs docetaxel (12.2 vs 9.4 months; HR 0.73; p=0.002). In contrast, median PFS was longer with docetaxel (4.2 vs 2.3 months; HR 0.92; p=0.39, respectively) [36]. These results were obtained regardless of the PD-L1 tumor status, which may influence the response rate and the duration of response to ICI. Nivolumab monotherapy was also approved in previously treated metastatic NSCLC, irrespective of PD-L1 expression.

Atezolizumab

OAK phase III clinical trial compared atezolizumab vs docetaxel in second-line treatment of advanced NSCLC. OS was significantly improved with atezolizumab vs docetaxel (13.8 vs 9.6 months; HR 0.73; p=0.0003). OS was also improved with atezolizumab both in PD-L1 positive and PD-L1 negative groups (15.7 vs 10.3 months; HR 0.74; p= 0.0102 and 12.6 vs 8.9 months HR 0.75; p=0.0215, respectively). Interestingly, in tumors harboring *EGFR* activating mutation subgroup (n=42 and 43, respectively), median OS was not in favor of atezolizumab (10.5 vs 16.2 months; HR 1.24), even though patients recruited in this study with *EGFR* mutated tumors were required to have received approved targeted therapy [37]. These data are consistent with those previously reported for other ICI therapy [36] and suggest a decreased immunogenicity of these tumors. The long-term survival analyses confirm the benefit of atezolizumab vs docetaxel after >2 years of follow-up [38]. Atezolizumab is approved for the treatment of metastatic NSCLC with disease progression after chemotherapy.

Durvalumab

PACIFIC phase III clinical trial compared durvalumab vs placebo for consolidation therapy in patients with unresectable stage III NSCLC that did not progress after concurrent chemo-radiotherapy, regardless of PD-L1 status. Median PFS was longer with durvalumab vs placebo (16.8 vs 5.6 months; HR 0.52; p<0.001) while ORR and 24-months OS rate were higher (28.4% vs 16%; p<0.001 and 66.3% vs 55.6%, p=0.005, respectively) [39,40]. Based on these results, FDA and EMA approved durvalumab as monotherapy for unresectable PD-L1 positive NSCLC that had not progressed following platinum-based chemoradiation therapy.

Avelumab

Avelumab is an anti-PD-L1 antibody for which the results of the first phase 3 clinical trial were published in 2018 (JAVELIN 200 Lung trial). Avelumab was evaluated vs docetaxel as second-line treatment in patients after progression with a platinum-containing doublet (n=792). In patients with PD-L1 ≥1% tumors, median OS was not significantly longer between the avelumab and docetaxel groups (11.4 vs 10.3 months; HR 0.90; p=0.16). In contrast, median OS was longer with avelumab in the subgroups with PD-L1 ≥50% or PD-L1 ≥80% tumors (13.6 vs 9.2 months; HR 0.67; p=0.0052 and 17.1 vs 9.3 months; HR 0.59; p=0.0022, respectively). These results suggest that avelumab could improve OS in patients harboring a high PD-L1 expression tumor. There is no FDA or EMA approved indication for avelumab in NSCLC at this time [41].

4- Discussion on the current indications of ICI

There is now a significant place for immunotherapy both in first- and second-line treatment of advanced NSCLC. ICI should be used as soon as possible in the therapeutic sequence, even in first line treatment in all FDA/EMA approved indications. However, a tumor molecular analysis has to be performed prior to starting treatment to identify the oncogenic driver. It is now clearly established that mutated *EGFR* or *ALK*-rearranged tumors may benefit from targeted therapy (*i.e.* EGFR tyrosine kinase inhibitors and ALK inhibitors). These tumors are associated with a cold tumor immune microenvironment despite a high rate of increased PD-L1 expression, and will not benefit from ICI treatment. ICI remains an option for these patients after progression with targeted therapy and standard chemotherapies. Moreover, ICI in first line treatment of tumors harboring targetable oncogenic drivers such as *BRAF* mutation, *ERBB2* exon 20 duplication, *MET* exon 14 skipping or *ROS1* and *RET* rearrangement should be discussed by the tumor advisory board (TAB). Different studies have shown that first line ICI alone or ICI - chemotherapy combos significantly enhance PFS and OS compared with standard chemotherapy. In KEYNOTE-024 clinical trial (*i.e.* pembrolizumab vs chemotherapy in PD-L1 ≥50% tumors), the ORR in the pembrolizumab group was 44.8% whereas ORR was 61.4% in KEYNOTE-189 (*i.e.* pembrolizumab plus chemo in PD-L1 >50% tumors). Median OS has not yet been reached for KEYNOTE-189 trial and could therefore not be compared to the 30 months evaluated in the KEYNOTE-024 results. Nevertheless, the best therapeutic approach still remains unclear. Further clinical trials are needed to compare these two therapeutic approaches. In case of rapid disease progression, the ICI - chemotherapy

combo could be the best choice in first line treatment, due to the faster speed of action of chemotherapy as compared with ICI.

IV - Molecular ICI resistance profile

Despite durable response rates observed with ICI therapies, the majority of patients fail to benefit from the treatment (primary resistance) and some responders ultimately relapse after initially responding to therapy (acquired resistance). Interactions between cancer cells and the immune system are dynamic and constantly evolving. As a consequence, adaptive resistance consists of mechanisms that lead to the protection of the tumor by facilitating its adaption to the immune response [42]. Although resistance to ICI may manifest clinically at different times, in many cases similar or overlapping mechanisms are involved. Here we present known resistance mechanisms categorized as tumor-intrinsic (markers present at the tumor-cell level) or tumor-extrinsic mechanisms (markers present at the immune system level or at the microenvironment level) (figure 1). Various tumor-intrinsic mechanisms have recently been identified in IT resistance: i) lack of T cell responses due to the loss of tumor antigen expression; ii) loss of MHC class I expression; iii) loss of interferon-gamma (IFN-γ) signaling; iv) modulation of canonical signaling pathways such as WNT/β-catenin, PI3K/Akt/mTOR and/or mitogen-activated protein kinase (MAPK) pathways v) *STK11/KRAS* co-mutations. Extrinsic resistance mechanisms involve components other than tumor cells within the tumor microenvironment: immune cells such as regulatory T cells (Tregs), myeloid derived suppressor cells (MDSCs), M2 macrophages; angiogenesis; exosomes and microbiomes.

Tumor-cell intrinsic mechanisms of ICI resistance

Tumor-cell intrinsic mechanisms may exist at ICI treatment onset or may evolve later. These mechanisms include the absence of antigenic protein, of antigen presentation or signaling pathway regulation in tumor cells.

One of the main causes of ICI resistance is a low DNA mutation rate leading to low neo-antigen generation with a lack of T-cell recognition [43]. In contrast, it is now well established that tumor instability (*i.e.* MSH-H phenotype) triggers neo-antigen generation and is asociated with a higher response rate in ICI treatment. Moreover, tumor instability may lead to *POLD1* or *POLE* mutations, especially in the exonucleasic domain, which cause the acquisition of the recently describe ultra-

hypermutated tumor phenotype. These tumors remain rare in NCSLC but are described as notably sensitive to ICI therapy.

Genetic deletions, mutations or epigenetic changes leading to loss of expression of the mutational neo-antigens presented by MHC molecules, may result in acquired resistance to ICI therapy. Alternatively, loss of MHC class I expression will affect the T-cell recognition of tumor antigens. One important mechanism is the reduced expression or function loss of β2-microglobulin (B2M), which is an essential factor of class 1 MHC folding and transport to the tumor cell surface. B2M mutations or loss of heterozygosity (LoH) could then lead to the loss of MHC class I and to subsequent defective antigen processing [44]. Epigenetic events have also been found to modify MHC expression on tumor cell surface, and could play a critical role in modifying the tumor cell immunogenicity [45].

The loss of IFN-γ sensitivity of cancer cells results in the decreased expression of MHC, PD-L1 and chemokines. This in turn results in a reduced T-cell recognition of cancer cells and decreases further antitumor T-cell recruitment, ultimately leading to tumor cells escaping. One mechanism of tumor insensitivity to interferons could be a genetic or epigenetic defect in IFN-γ receptors JAK1/JAK2 (*e.g.* truncating mutation *JAK1* p.(Gln503*) or splice variant *JAK2* c.1641+2T>G p.(Phe547_splice)), in transcription factor STAT or in other genes involved in the IFN-γ signaling pathway [46].

Activation of oncogenic intracellular pathways such as Wnt/β-catenin signaling can lead to genetic T-cell exclusion, and correlate with non-inflamed tumors with low $CD8^+$ infiltrate. β-catenin signaling activation may be due to various mechanisms including gene mutations, amplification or the DNA methylation of molecules related to this pathway. Molecular alterations in this pathway mostly involve *CTNNB1* exon 3 (*i.e.* mutation of phosphorylation sites or deletions), *APC, AXIN1 or GSK3*-β. This results in a stabilization of β-catenin complex and an activation of Wnt/β-catenin pathway. Among Wnt proteins, Wnt1 has been described as inversely correlated with T cell abundance in NSCLC. The subsequent mechanism may be linked to the tumor cell secretion of Wnt1 that may suppress dendritic cell chemokine secretion [47]. Thereby, the activation of β-catenin signaling disrupts the T-cell responses that make up the ICI resistance mechanism [48].

The oncogenic PI3K-Akt-mTOR signaling pathway activation plays a role in the regulation of PD-L1 both *in-vitro* and *in-vivo* in NSCLC [49]. Activating mutations of *PIK3CA* (*e.g.* p.(His1047Arg), p.(Glu542X), p.(Glu545X)) or loss of *PTEN* expression through deletion,

truncating mutations and LoH) are involved in the resistance to ICI therapy. These mutations increase the expression of immunosuppressive cytokines and is also associated with a reduced $CD8^+$ infiltrate in different tumor models [50–52]. *PTEN* alterations are commonly found in the molecular profile of NSCLC but not all alterations have the same clinical impact. PTEN expression is regulated at multiple genetic and epigenetic levels, which are not yet all evaluated in daily practice [53]. Moreover, activating *AKT* mutations remain uncommon in NSCLC but have also been described to activate the downstream pathway [49]. This provides the rationale to combine ICI with inhibitors of upstream PI3K or downstream VEGF, to enhance anti-tumor efficacy [31].
MAPK is a major signaling pathway involved in NSCLC carcinogenesis through regulation of cell proliferation, migration, differentiation and survival. Some studies have shown that the activation of MAPK pathway could promote immune-evasion [54] and that the combination of ICI with MAPK pathway inhibitors could be useful [55].
STK11 (LKB1) alterations are not uncommon in NSCLC, especially in association with *KRAS* mutations [56]. *KRAS/STK11* co-mutations showed significantly lower ORR to ICI as compared with *KRAS* mutated tumors in lung adenocarcinoma (7.4% vs 28.6%, $p<0.001$). Moreover, *KRAS/STK11* co-mutations showed shorter PFS and OS as compared with *KRAS* or *STK11* mutated tumors (1.8 and 6.4 months vs 2.7 and 16.0 months; HR 1.87, $p<0.001$ and HR 1.99, $p=0.0015$, respectively) in a cohort of 174 patients [57]. *STK11* impairment appears as an important predictive cause of primary resistance to ICI in NSCLC [58] and seems associated with a decreased expression of PD-L1 in tumor cells [57].

Tumor-cell extrinsic mechanisms of resistance to ICI

Tumor-cell extrinsic mechanisms may also account for primary and/or acquired resistance to ICI. These mechanisms involve the lack of molecules essential for T-cell induction within the tumor environment or the presence of immunosuppressive cells. T-cell absence with tumor antigen specific TCRs infiltrating the tumor (T-cell non inflamed) could be due to a defect in the T-cell induction pathway, caused by insufficient immunogenic tumor antigens due to low tumor mutation burden [59]. Insufficient chemokines or cytokines arising from gene deletion or reduced gene expression may also lower the antigen-presenting cell recruitment into tumors and subsequent T-cell effector expansion.

Immunosuppressive TGF-β inhibits the induction of tumor-antigen specific $CD8^+$ in lymph nodes and in subsequent $CD8^+$ infiltration of the tumor through dendritic cell function impairment and the recruitment of immunosuppressive cells such as myeloid derived suppressor cells (MDSC) and Tregs [60]. TGF-β may induce a mesenchymal tumor microenvironment, characterized by a gene expression pattern related to angiogenesis, wound healing and epithelial-to-mesenchymal transition [61].

Tumor immune escape is also related to tumor angiogenesis, which depends on immunosuppressive microenvironment. VEGF and its receptor VEGFR2 are predominant targets for the development of anti-angiogenic agents in NSCLC. Anti-angiogenic therapies targeting vascular endothelial growth factor VEGF receptors (VEGFR) can significantly improve efficacy of ICI. Preclinical and clinical studies demonstrate the synergistic effects of anti-angiogenic agents and ICI therapy [62].

ICI resistance is associated with a release of exosomal PD-L1 that inhibits IFN-γ secretion cells and impairs immune functions by reducing cytokine production and inducing apoptosis in $CD8^+$ T cells [63].

Finally, alterations in the gut microbiome have been associated with resistance to ICI [64]. The immune system could be modulated by the microbiome, through innate immunity activation [65]. Microbiome-induced immunomodulatory could be used to enhance ICI efficacy.

These results all confirm the importance of the molecular analysis of tumor samples in diagnosing advanced NSCLC, but their potential use before and during ICI therapy. Identifying these molecular patterns in canonical pathways may be essential for monitoring and adaptating targeted therapy in case of IT resistance.

V – Outlook on resistance prediction to ICI

Predicting ICI resistance remains a challenge, as there is a lack of validated biomarkers to monitor treatment response. Preliminary data suggest that serial liquid biopsies for monitoring cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), circulating tumor cells (CTC) and/or exosomes dynamics could serve as non-invasive useful markers of response to treatment, including ICI [66]. Liquid biopsies represent an important source of cfDNA/ctDNA. Detection of targeted mutations in cfDNA/ctDNA, assessment of blood tumor mutation burden (bTMB), quantification of cfDNA/ctDNA or copy number instability are currently under investigation for the monitoring of

ICI sensitivity/response and promising results have been obtained. For example, the identification of *STK11* or *PTEN* mutations in cfDNA/ctDNA may help to identify ICI non-responding patients at treatment initiation [67]. A high bTMB has been demonstrated to be predictive of immunotherapy sensitivity in NSCLC patients [68]. NGS analysis with a comprehensive cancer gene panel could be an interesting indicator to identify clonal evolution and to detect early relapse [69].

CTC are presumably derived from all tumor sites and should consequently give a better global representation of PD-L1 expression than one tissue sample. Detection of PD-L1 expression on CTC has been shown to correlate with PD-L1 status on tumor tissue and could be a potential marker to monitor ICI response [70]. However, contradictory results have been reported and several technical pitfalls must be overcome, mostly related to CTCs usually being very rare and the use of different isolation methods (antigen-dependent or -independent), leading to enrichment of different CTC populations [71,72]. The use of CTC detection as a biomarker in clinical practice needs further standardization.

In recent studies, exosomes were isolated from plasma and their PD-L1 mRNA level was reported to be associated with response to ICI therapy in melanoma and NSCLC patients [73]. Patients responding to nivolumab and pembrolizumab had higher exosomal PD-L1 mRNA expression at the beginning of the treatment compared with patients that did not respond. Moreover, a decreased exosomal PD-L1 mRNA expression was observed after 2 months of treatment for the responders while it remained unchanged in non-responders. PD-L1 mRNA level in exosomes isolated from bio-fluids may represent a reliable predictive biomarker of immunotherapy sensitivity.

V - Conclusion

IT use in NSCLC is growing. The administration of ICI in daily practice has notably increased PFS and OS in patients with advanced NSCLC, while maintaining a good quality of life. This has given rise to many potential targets being extensively studied to enhance the number of available therapies. However, IT does not benefit the majority of patients, which suggests the existence of resistance mechanisms. Numerous studies have shown that specific molecular patterns could be involved in IT resistance mainly through the alteration of the canonical cell signaling pathways. Tremendous efforts in translational research are needed to examine the various parameters involved, either at the tumor-cell level or the microenvironment level (macrophages, fibroblasts,

microbiota and etc.). Molecular analysis of tumors or liquid biopsies could highlight molecular profiles predicting a part of the IT response, in addition to the commonly used PD-L1 expression. For resistant ICI patients, other IT could target or use cytokines, tumor-directed antibodies, antibody-drug conjugates, chimeric antigen receptor (CAR) T cells therapy, cancer vaccines, oncolytic viruses or rotavirus vaccines [18,74,75].

Table I: Current and promising targets of IT in NSCLC

Target	Therapeutic agents	Reference
ATP synthase beta subunit (ATPB)	4E7 (McAb4E7)	[76]
CD317	HM1,24	[77]
Cytotoxic T-lymphocyte associated antigen 4 (CTLA-4)	Ipilimumab	[78,79]
Cystein-rich angiogenic inducer-61 (CYR-61)	Anti-CR-61	[80]
EGF	CIMAvaxEGF	[79]
Folate receptor alpha (FRA)		[81]
IL-17-Th1/Th17		[82]
LUNX		[83]
Melanoma-associated-antigen A3 (MAGE-A3)	MAGRIT	[79]
MUC1 (mucin-1)	Tecemotide (BLP-25), TG4010	[78,79,84]
NeuGc-containing gangliosides	Racotumomab	[85]
New York esophageal squamous cell carcinoma 1 (NY-ESO-1)		[86,87]
PD-1 (CD279)	Nivolumab, pembrolizumab, lambrolizumab	[78,79]
PD-L1 (B7-H1/CD274)	BMS-9365559 (MDX-1105), atezolizumab, avelumab, envafolimab, CK-301, CS-1001, BGB-A333	[74,78,88,89]
scavenger receptor MARCO		[90]
Survivin-2B		[91]
VEGF	Bevacizumab	[92]
VEGFR-2	Ramucirumab	[92]
VISTA protein		[93]
Whole cell vaccine transfecting tumor cells with a TGF-β2 gene	Belagenpumatucel-L(Lucanix)	[79]

Table II: Summary of the main clinical trial and FDA/EMA approved ICI in non-squamous NSCLC

Study	Ref.	Purpose	N	Results	Approved treatment option
PEMBROLIZUMAB					
KEYNOTE-024 NCT02142738 (2016)	[21,22]	First line pembrolizumab vs chemotherapy PD-L1 ≥50%	305	Median PFS was 10.3 months with pembrolizumab vs 6.0 months with chemotherapy (p<0.001). The RR was 44.8% with pembrolizumab vs 27.8% with chemotherapy. Median OS was 30.0 months with pembrolizumab vs 14.2 months with chemotherapy (p<0.002).	Pembrolizumab in first line treatment of advanced NSCLC; PD-L1≥50%
KEYNOTE-042 NCT02220894 (2019)	[23]	First line pembrolizumab vs chemotherapy PD-L1 ≥1%	1274	Median PFS was 7.1 months with pembrolizumab vs 6.4 months with chemotherapy in PD-L1 ≥50% population. Median OS was 16.7 months with pembrolizumab vs 12.1 months with chemotherapy (p<0.0018) in PD-L1 ≥1% population. Median OS was 13.4 months with pembrolizumab vs 12.1 months with chemotherapy in PD-L1 expression of 1-49% population.	Pembrolizumab in first line treatment of advanced NSCLC; PD-L1≥1%
KEYNOTE-189 NCT02578680 (2018)	[26,27]	First line pemetrexed and platinum-based chemotherapy +/- pembrolizumab	616	Estimated OS rate at 12 months was 69.2% in the chemotherapy- pembrolizumab group vs 49.4% in the chemotherapy group (p<0.001). The benefit in 12-month OS rate was observed even in tumors with <1% PD-L1 expression (61.7% vs 52.2%; HR 0.59). After a 18.7-month median follow up, PFS and OS were longer with pembrolizumab plus chemotherapy irrespective of PD-L1 tumor expression (9.0 vs 4.9 months; HR 0.48 and 22.0 vs 10.7 months; HR 0.56; p<0.00001, respectively).	Pembrolizumab + pemetrexed /platinum-based chemotherapy in first line treatment of advanced non-squamous NSCLC
KEYNOTE-010 NCT01905667 (2016)	[34,35]	Pembrolizumab vs docetaxel for previously treated PD-L1 positive (≥1%) NSCLC	1034	Median PFS and OS were longer with pembrolizumab vs chemotherapy (5.2 vs 4.1 and 17.3 vs 8.2 months, p<0.0001, respectively) in PD-L1 ≥50% population. In PD-L1 ≥1% population, no significant difference was noticed in PFS between the two groups. OS was significantly longer with pembrolizumab (12.7 vs 8.5 months; HR 0.61; p<0.0001).	Pembrolizumab in previously treated advanced NSCLC; PD-L1≥1%
NIVOLUMAB					

CheckMate 227 NCT02477826 (2018)	[28,29]	Multipart first-line phase 3 trial: - PD-L1 ≥ 1%: nivolumab plus ipilimumab vs nivolumab monotherapy vs chemotherapy - PD-L1 <1%: nivolumab plus ipilimumab vs nivolumab plus chemotherapy vs chemotherapy	1739	In high TMB subgroup, the 1-year PFS rate and median PFS were longer with nivolumab plus ipilimumab vs chemotherapy (42.6% vs 13.2% and 7.2 vs 5.5 months respectively; HR 0.58 p<0.001). Regardless of the PD-L1 expression level, median OS was also longer with nivolumab plus ipilimumab vs chemotherapy (17.1 vs 13.9 months; HR 0.73).	
CheckMate 57 NCT01673867 (2015)	[36]	Nivolumab vs docetaxel for previously treated non-squamous NSCLC	582	Median PFS was 2.3 months with nivolumab vs 4.2 months with docetaxel. Median OS was 12.2 months with nivolumab vs 9.4 months with docetaxel (p=0.002). RR was 19% with nivolumab vs 12% with docetaxel (p=0.02).	Nivolumab in previously treated advanced NSCLC
ATEZOLIZUMAB					
IMpower 150 NCT02366143 (2018)	[31]	Multipart first-line phase 3 trial in metastatic nonsquamous NSCLC: - ACP: atezolizumab plus carboplatin plus paclitaxel - BCP : bevacizumab plus carboplatin plus paclitaxel - ABCP: atezolizumab plus BCP	ACP: 348 BCP: 336 ABCP: 356	Median PFS and OS were longer in the ABCP group vs the BCP group (8.3 vs 6.8 months, p<0.001 and 19.2 vs 14.7 months, p=0.02, respectively) In patients with EGFR or ALK genetic alternation subgroup (n=108), median PFS was also longer in the ABCP group vs the BCP group (9.7 vs 6.1 months, respectively, HR=0.59).	Atezolizumab + bevacizumab + carboplatin + paclitaxel in first line treatment of advanced non-squamous NSCLC
IMpower 130 NCT02367781 (2019)	[33]	First-line carboplatin + nab-paclitaxel +/- atezolizumab	723	Median PFS was 7.0 months in the atezolizumab plus chemotherapy group vs 5.5 months with chemotherapy alone (HR 0.64; p<0.0001) Median OS was 18.6 months in the atezolizumab plus chemotherapy group vs 13.9 months with chemotherapy alone (HR 0.79; p=0.033)	Atezolizumab + carboplatin + nab-paclitaxel in first line treatment of advanced non-squamous NSCLC
OAK NCT02008227 (2017)	[37,38]	Atezolizumab vs docetaxel for previously treated NSCLC	850	OS was 13.8 months with atezolizumab vs 9.6 months with docetaxel (HR 0.73; p=0·0003). OS was improved both in PD-L1 positive and negative populations. OS was similarly improved in squamous and non-squamous histological subgroups.	Atezolizumab in previously treated advanced NSCLC
DURVALUMAB					
PACIFIC NCT02125461 (2017)	[39,40]	Durvalumab vs placebo in unresectable stage III NSCLC that did not progress after concurrent chemo-radiotherapy	709	Median PFS was 16.8 months with durvalumab vs 5.6 months with placebo (p<0.001) while the 18-month PFS rate was 44.2% vs 27%, respectively.	Durvalumab in PD-L1 positive NSCLC that had not progressed

				The ORR was higher with durvalumab than with placebo (28.4% vs. 16.0%; P<0.001) The 24-month OS rate was 66.3% with durvalumab vs 55.6% with placebo (p=0.005). OS was longer with durvalumab vs placebo (HR 0.68; p=0.0025).	following platinum‐based chemoradiation therapy
AVELUMAB					
JAVELIN Lung 200 NCT02395172 (2018)	[41]	Avelumab vs docetaxel for previously treated NSCLC	792	In patients with PD-L1 ≥1% tumors, median OS was not significantly longer between the avelumab and docetaxel groups (11.4 vs 10.3 months; HR 0.90; p=0.16)	

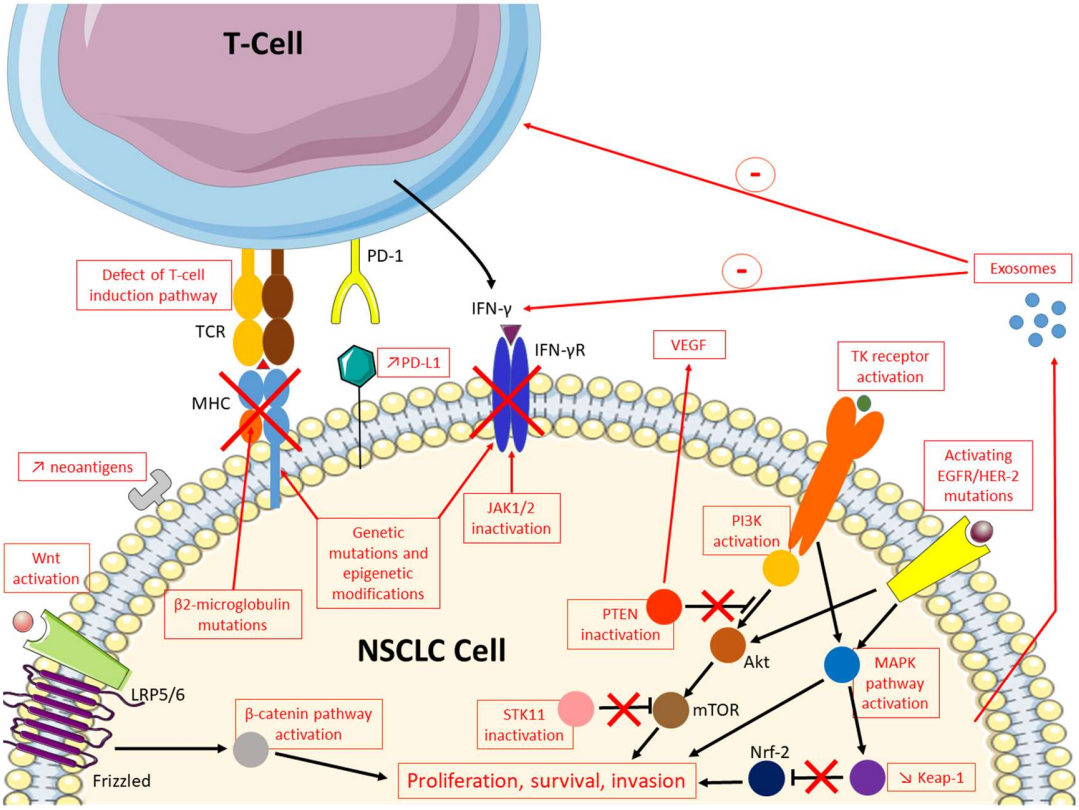

Figure Legend

Figure 1: Mechanisms of ICI resistance in NSCLC.

Genetic alterations and epigenetic modifications alter components of the antigen presentation system. Loss of β2-microglobunin expression results in the impaired cell surface expression of MHC class I, which in turn impairs antigen presentation to cytotoxic T cells. The mutational activation of phosphatidylinositol 3-kinase (PI3K) or the loss of PTEN is associated with the activation of the PI3K-Akt-mTOR signaling pathway leading to cell proliferation, survival and invasion. Loss of PTEN also increases VEGF levels. Mutations resulting in the loss of function of JAK1 and JAK2 impair the IFN-γR signaling pathway, resulting in the loss of IFN-γ anti-tumor effects. Activating mutations in Wnt/β-catenin pathway result in cell proliferation and survival. Activated mutations of EGFR or HER-2 induce Akt/mTOR and MAPK pathways resulting in cell proliferation, survival and invasion. Keap1/Nrf2 pathway promotes survival in the presence of multiple inhibitors targeting the RTK/Ras/MAPK pathway. Loss of Keap1 increases Nrf2 level and favored cell survival. ICI resistance increases exosome release by tumor cell, leading to a decrease in IFN-γ, and impairs immune functions by reducing cytokine production and inducing apoptosis in $CD8^+$ T-cells.

References

1. Lee H, Lee S, Heo Y-S. Molecular Interactions of Antibody Drugs Targeting PD-1, PD-L1, and CTLA-4 in Immuno-Oncology. *Molecules*. 24(6), 1190 (2019).
2. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 359(6382), 1350–1355 (2018).
3. Buchbinder EI, Desai A. CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. *American Journal of Clinical Oncology*. 39(1), 98–106 (2016).
4. Boyiadzis MM, Kirkwood JM, Marshall JL, Pritchard CC, Azad NS, Gulley JL. Significance and implications of FDA approval of pembrolizumab for biomarker-defined disease. *Journal for ImmunoTherapy of Cancer* [Internet]. 6(1) (2018). Available from: http://jitc.bmj.com/lookup/doi/10.1186/s40425-018-0342-x.
5. Seidel JA, Otsuka A, Kabashima K. Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations. *Front. Oncol.* 8, 86 (2018).
6. Dubois M, Ardin C, André F, Scherpereel A, Mortier L. L'immunothérapie, une révolution en oncologie: Revue de l'efficacité des inhibiteurs de points de contrôle immunitaire. *médecine/sciences*. 35(12), 937–945 (2019).
7. Prelaj A, Tay R, Ferrara R, Chaput N, Besse B, Califano R. Predictive biomarkers of response for immune checkpoint inhibitors in non–small-cell lung cancer. *European Journal of Cancer*. 106, 144–159 (2019).
8. Willis C, Fiander M, Tran D, *et al.* Tumor mutational burden in lung cancer: a systematic literature review. *Oncotarget* [Internet]. 10(61) (2019). Available from: http://www.oncotarget.com/fulltext/27287.
9. Wang C, Liang H, Lin C, *et al.* Molecular Subtyping and Prognostic Assessment Based on Tumor Mutation Burden in Patients with Lung Adenocarcinomas. *International Journal of Molecular Sciences*. 20(17), 4251 (2019).
10. Lv Y, Huang Z, Lin Y, *et al.* MiRNA expression patterns are associated with tumor mutational burden in lung adenocarcinoma. *OncoImmunology*. 8(10), e1629260 (2019).
11. Wu Y, Xu J, Du C, *et al.* The Predictive Value of Tumor Mutation Burden on Efficacy of Immune Checkpoint Inhibitors in Cancers: A Systematic Review and Meta-Analysis. *Frontiers in Oncology* [Internet]. 9 (2019). Available from: https://www.frontiersin.org/article/10.3389/fonc.2019.01161/full.
12. Krieg C, Nowicka M, Guglietta S, *et al.* High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. *Nature Medicine*. 24(2), 144–153 (2018).
13. Rotow J, Bivona TG. Understanding and targeting resistance mechanisms in NSCLC. *Nature Reviews Cancer*. 17(11), 637–658 (2017).
14. Peranzoni E, Donnadieu E. Improving efficacy of cancer immunotherapy through targeting of macrophages. *Human Vaccines & Immunotherapeutics*. 15(1), 189–192 (2019).
15. Damotte D, Warren S, Arrondeau J, *et al.* The tumor inflammation signature (TIS) is associated with anti-PD-1 treatment benefit in the CERTIM pan-cancer cohort. *Journal of Translational Medicine* [Internet]. 17(1) (2019). Available from: https://translational-medicine.biomedcentral.com/articles/10.1186/s12967-019-2100-3.
16. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. *Blood*. 131(1), 58–67 (2018).
17. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: Co-inhibitory Receptors with Specialized Functions in Immune Regulation. *Immunity*. 44(5), 989–1004 (2016).

18. Andrews LP, Marciscano AE, Drake CG, Vignali DAA. LAG3 (CD223) as a cancer immunotherapy target. *Immunological Reviews*. 276(1), 80–96 (2017).
19. Muller S, Victoria Lai W, Adusumilli PS, *et al.* V-domain Ig-containing suppressor of T-cell activation (VISTA), a potentially targetable immune checkpoint molecule, is highly expressed in epithelioid malignant pleural mesothelioma. *Modern Pathology*. 33(2), 303–311 (2020).
20. Hahn AW, Gill DM, Pal SK, Agarwal N. The future of immune checkpoint cancer therapy after PD-1 and CTLA-4. *Immunotherapy*. 9(8), 681–692 (2017).
21. Reck M, Rodríguez-Abreu D, Robinson AG, *et al.* Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. *New England Journal of Medicine*. 375(19), 1823–1833 (2016).
22. Reck M, Rodríguez–Abreu D, Robinson AG, *et al.* Updated Analysis of KEYNOTE-024: Pembrolizumab Versus Platinum-Based Chemotherapy for Advanced Non–Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score of 50% or Greater. *Journal of Clinical Oncology*. 37(7), 537–546 (2019).
23. Mok TSK, Wu Y-L, Kudaba I, *et al.* Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *The Lancet*. 393(10183), 1819–1830 (2019).
24. Carbone DP, Reck M, Paz-Ares L, *et al.* First-Line Nivolumab in Stage IV or Recurrent Non–Small-Cell Lung Cancer. *N Engl J Med*. 376(25), 2415–2426 (2017).
25. Spigel D. IMPOWER110: INTERIM OVERALL SURVIVAL (OS) ANALYSIS OF A PHASE III STUDY OF ATEZOLIZUMAB (ATEZO) VS PLATINUM-BASED CHEMOTHERAPY (CHEMO) AS FIRST-LINE (1L) TREATMENT (TX) IN PD-L1–SELECTED NSCLC. Annals of Oncology, v851–v934 (2019).
26. Gandhi L, Rodríguez-Abreu D, Gadgeel S, *et al.* Pembrolizumab plus Chemotherapy in Metastatic Non–Small-Cell Lung Cancer. *New England Journal of Medicine*. 378(22), 2078–2092 (2018).
27. Gadgeel S, Rodríguez-Abreu D, Speranza G, *et al.* Updated Analysis From KEYNOTE-189: Pembrolizumab or Placebo Plus Pemetrexed and Platinum for Previously Untreated Metastatic Nonsquamous Non–Small-Cell Lung Cancer. *JCO*. , JCO.19.03136 (2020).
28. Hellmann MD, Ciuleanu T-E, Pluzanski A, *et al.* Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. *New England Journal of Medicine*. 378(22), 2093–2104 (2018).
29. Hellmann MD, Paz-Ares L, Bernabe Caro R, *et al.* Nivolumab plus Ipilimumab in Advanced Non–Small-Cell Lung Cancer. *New England Journal of Medicine*. 381(21), 2020–2031 (2019).
30. Socinski M, Velcheti V, Mekhail T, *et al.* Final efficacy results from B-F1RST, a prospective phase II trial evaluating blood-based tumour mutational burden (bTMB) as a predictive biomarker for atezolizumab (atezo) in 1L non-small cell lung cancer (NSCLC). *Annals of Oncology*. 30, v919–v920 (2019).
31. Socinski MA, Jotte RM, Cappuzzo F, *et al.* Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. *New England Journal of Medicine*. 378(24), 2288–2301 (2018).
32. Socinski MA, Jotte RM, Cappuzzo F, *et al.* Overall survival (OS) analysis of IMpower150, a randomized Ph 3 study of atezolizumab (atezo) + chemotherapy (chemo) ± bevacizumab (bev) vs chemo + bev in 1L nonsquamous (NSQ) NSCLC. *Journal of Clinical Oncology*. 36(15_suppl), 9002–9002 (2018).
33. West H, McCleod M, Hussein M, *et al.* Atezolizumab in combination with carboplatin plus

nab-paclitaxel chemotherapy compared with chemotherapy alone as first-line treatment for metastatic non-squamous non-small-cell lung cancer (IMpower130): a multicentre, randomised, open-label, phase 3 trial. *The Lancet Oncology*. 20(7), 924–937 (2019).
34. Herbst RS, Baas P, Kim D-W, *et al.* Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *The Lancet*. 387(10027), 1540–1550 (2016).
35. Herbst RS. Long-term follow-up in the KEYNOTE-010 study of pembrolizumab (pembro) for advanced NSCLC, including in patients (pts) who completed 2 years of pembro and pts who received a second course of pembro. , x39-X43 (2018).
36. Borghaei H, Paz-Ares L, Horn L, *et al.* Nivolumab versus Docetaxel in Advanced Nonsquamous Non–Small-Cell Lung Cancer. *New England Journal of Medicine*. 373(17), 1627–1639 (2015).
37. Rittmeyer A, Barlesi F, Waterkamp D, *et al.* Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *The Lancet*. 389(10066), 255–265 (2017).
38. von Pawel J, Bordoni R, Satouchi M, *et al.* Long-term survival in patients with advanced non–small-cell lung cancer treated with atezolizumab versus docetaxel: Results from the randomised phase III OAK study. *European Journal of Cancer*. 107, 124–132 (2019).
39. Antonia SJ, Villegas A, Daniel D, *et al.* Durvalumab after Chemoradiotherapy in Stage III Non–Small-Cell Lung Cancer. *New England Journal of Medicine*. 377(20), 1919–1929 (2017).
40. Antonia SJ, Villegas A, Daniel D, *et al.* Overall Survival with Durvalumab after Chemoradiotherapy in Stage III NSCLC. *New England Journal of Medicine*. 379(24), 2342–2350 (2018).
41. Barlesi F, Vansteenkiste J, Spigel D, *et al.* Avelumab versus docetaxel in patients with platinum-treated advanced non-small-cell lung cancer (JAVELIN Lung 200): an open-label, randomised, phase 3 study. *The Lancet Oncology*. 19(11), 1468–1479 (2018).
42. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell*. 168(4), 707–723 (2017).
43. Gubin MM, Zhang X, Schuster H, *et al.* Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature*. 515(7528), 577–581 (2014).
44. Gettinger S, Choi J, Hastings K, *et al.* Impaired HLA Class I Antigen Processing and Presentation as a Mechanism of Acquired Resistance to Immune Checkpoint Inhibitors in Lung Cancer. *Cancer Discovery*. 7(12), 1420–1435 (2017).
45. Olino K, Park T, Ahuja N. Exposing Hidden Targets: Combining epigenetic and immunotherapy to overcome cancer resistance. *Seminars in Cancer Biology* [Internet]. (2020). Available from: https://linkinghub.elsevier.com/retrieve/pii/S1044579X2030002X.
46. Zaretsky JM, Garcia-Diaz A, Shin DS, *et al.* Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. *New England Journal of Medicine*. 375(9), 819–829 (2016).
47. Kerdidani D, Chouvardas P, Arjo AR, *et al.* Wnt1 silences chemokine genes in dendritic cells and induces adaptive immune resistance in lung adenocarcinoma. *Nature Communications* [Internet]. 10(1) (2019). Available from: http://www.nature.com/articles/s41467-019-09370-z.
48. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature*. 523(7559), 231–235 (2015).
49. Lastwika KJ, Wilson W, Li QK, *et al.* Control of PD-L1 Expression by Oncogenic Activation of the AKT–mTOR Pathway in Non–Small Cell Lung Cancer. *Cancer Research*. 76(2), 227–238 (2016).

50. Peng W, Chen JQ, Liu C, *et al.* Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy. *Cancer Discovery*. 6(2), 202–216 (2016).
51. George S, Miao D, Demetri GD, *et al.* Loss of PTEN Is Associated with Resistance to Anti-PD-1 Checkpoint Blockade Therapy in Metastatic Uterine Leiomyosarcoma. *Immunity*. 46(2), 197–204 (2017).
52. Brandmaier A, Hou S-Q, Demaria S, Formenti SC, Shen WH. PTEN at the interface of immune tolerance and tumor suppression. *Frontiers in Biology*. 12(3), 163–174 (2017).
53. Gkountakos A, Sartori G, Falcone I, *et al.* PTEN in Lung Cancer: Dealing with the Problem, Building on New Knowledge and Turning the Game Around. *Cancers*. 11(8), 1141 (2019).
54. Loi S, Dushyanthen S, Beavis PA, *et al.* RAS/MAPK Activation Is Associated with Reduced Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancer: Therapeutic Cooperation Between MEK and PD-1/PD-L1 Immune Checkpoint Inhibitors. *Clinical Cancer Research*. 22(6), 1499–1509 (2016).
55. Liu L, Mayes PA, Eastman S, *et al.* The BRAF and MEK Inhibitors Dabrafenib and Trametinib: Effects on Immune Function and in Combination with Immunomodulatory Antibodies Targeting PD-1, PD-L1, and CTLA-4. *Clinical Cancer Research*. 21(7), 1639–1651 (2015).
56. Pécuchet N, Laurent-Puig P, Mansuet-Lupo A, *et al.* Different prognostic impact of *STK11* mutations in non-squamous non-small-cell lung cancer. *Oncotarget* [Internet]. 8(14) (2017). Available from: http://www.oncotarget.com/fulltext/6379.
57. Skoulidis F, Goldberg ME, Greenawalt DM, *et al. STK11/LKB1* Mutations and PD-1 Inhibitor Resistance in *KRAS* -Mutant Lung Adenocarcinoma. *Cancer Discovery*. 8(7), 822–835 (2018).
58. Kitajima S, Ivanova E, Guo S, *et al.* Suppression of STING Associated with LKB1 Loss in KRAS-Driven Lung Cancer. *Cancer Discovery*. 9(1), 34–45 (2019).
59. Rizvi NA, Hellmann MD, Snyder A, *et al.* Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. *Science*. 348(6230), 124–128 (2015).
60. Nakamura S, Yaguchi T, Kawamura N, *et al.* TGF-β1 in Tumor Microenvironments Induces Immunosuppression in the Tumors and Sentinel Lymph Nodes and Promotes Tumor Progression: *Journal of Immunotherapy*. 37(2), 63–72 (2014).
61. Hugo W, Zaretsky JM, Sun L, *et al.* Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell*. 165(1), 35–44 (2016).
62. Yi M, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment. *Molecular Cancer* [Internet]. 18(1) (2019). Available from: https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-019-0974-6.
63. Kim DH, Kim H, Choi YJ, *et al.* Exosomal PD-L1 promotes tumor growth through immune escape in non-small cell lung cancer. *Exp Mol Med*. 51(8), 1–13 (2019).
64. Gopalakrishnan V, Helmink BA, Spencer CN, Reuben A, Wargo JA. The Influence of the Gut Microbiome on Cancer, Immunity, and Cancer Immunotherapy. *Cancer Cell*. 33(4), 570–580 (2018).
65. Abt MC, Osborne LC, Monticelli LA, *et al.* Commensal Bacteria Calibrate the Activation Threshold of Innate Antiviral Immunity. *Immunity*. 37(1), 158–170 (2012).
66. Rossi G, Ignatiadis M. Promises and Pitfalls of Using Liquid Biopsy for Precision Medicine. *Cancer Research*. 79(11), 2798–2804 (2019).
67. Guibert N, Jones G, Beeler JF, *et al.* Targeted sequencing of plasma cell-free DNA to predict response to PD1 inhibitors in advanced non-small cell lung cancer. *Lung Cancer*. 137, 1–6 (2019).
68. Gandara DR, Paul SM, Kowanetz M, *et al.* Blood-based tumor mutational burden as a

predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nature Medicine*. 24(9), 1441–1448 (2018).
69. Li L, Wang Y, Shi W, *et al.* Serial ultra-deep sequencing of circulating tumor DNA reveals the clonal evolution in non-small cell lung cancer patients treated with anti-PD1 immunotherapy. *Cancer Medicine*. 8(18), 7669–7678 (2019).
70. Ilié M, Szafer-Glusman E, Hofman V, *et al.* Detection of PD-L1 in circulating tumor cells and white blood cells from patients with advanced non-small-cell lung cancer. *Annals of Oncology*. 29(1), 193–199 (2018).
71. Wu Z, Yang Z, Dai Y, Zhu Q, Chen L-A. Update on liquid biopsy in clinical management of non-small cell lung cancer. *OTT*. Volume 12, 5097–5109 (2019).
72. Hofman P, Heeke S, Alix-Panabières C, Pantel K. Liquid biopsy in the era of immuno-oncology: is it ready for prime-time use for cancer patients? *Annals of Oncology*. 30(9), 1448–1459 (2019).
73. Del Re M, Marconcini R, Pasquini G, *et al.* PD-L1 mRNA expression in plasma-derived exosomes is associated with response to anti-PD-1 antibodies in melanoma and NSCLC. *Br J Cancer*. 118(6), 820–824 (2018).
74. Akinleye A, Rasool Z. Immune checkpoint inhibitors of PD-L1 as cancer therapeutics. *Journal of Hematology & Oncology* [Internet]. 12(1) (2019). Available from: https://jhoonline.biomedcentral.com/articles/10.1186/s13045-019-0779-5.
75. Shekarian T, Sivado E, Jallas A-C, *et al.* Repurposing rotavirus vaccines for intratumoral immunotherapy can overcome resistance to immune checkpoint blockade. *Science Translational Medicine*. 11(515), eaat5025 (2019).
76. Lu Z, Song Q, Jiang S, *et al.* Identification of ATP synthase beta subunit (ATPB) on the cell surface as a non-small cell lung cancer (NSCLC) associated antigen. *BMC Cancer* [Internet]. 9(1) (2009). Available from: http://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-9-16.
77. Wang W, Nishioka Y, Ozaki S, *et al.* HM1.24 (CD317) is a novel target against lung cancer for immunotherapy using anti-HM1.24 antibody. *Cancer Immunology, Immunotherapy*. 58(6), 967–976 (2009).
78. Wurz GT, Kao C-J, Wolf M, DeGregorio MW. Tecemotide: An antigen-specific cancer immunotherapy. *Human Vaccines & Immunotherapeutics*. 10(11), 3383–3393 (2014).
79. Forde PM, Reiss KA, Zeidan AM, Brahmer JR. What Lies Within: Novel Strategies in Immunotherapy for Non-Small Cell Lung Cancer. *The Oncologist*. 18(11), 1203–1213 (2013).
80. Li X, Yuan N, Lin L, Yin L, Qu Y. Targeting cysteine-rich angiogenic inducer-61 by antibody immunotherapy suppresses growth and migration of non-small cell lung cancer. *Experimental and Therapeutic Medicine* [Internet]. (2018). Available from: http://www.spandidos-publications.com/10.3892/etm.2018.6274.
81. Wang Z, Shi H, Guo J, Li C. A current review of folate receptor alpha as a potential tumor target in non-small-cell lung cancer. *Drug Design, Development and Therapy*. , 4989 (2015).
82. Joerger M, Finn SP, Cuffe S, Byrne AT, Gray SG. The IL-17-Th1/Th17 pathway: an attractive target for lung cancer therapy? *Expert Opinion on Therapeutic Targets*. 20(11), 1339–1356 (2016).
83. Zheng X, Cheng M, Fu B, *et al.* Targeting LUNX Inhibits Non-Small Cell Lung Cancer Growth and Metastasis. *Cancer Research*. 75(6), 1080–1090 (2015).
84. Powell E, Chow LQ. BLP-25 liposomal vaccine: a promising potential therapy in nonsmall-cell lung cancer. *Expert Review of Respiratory Medicine*. 2(1), 37–45 (2008).
85. Segatori VI, Cuello HA, Gulino CA, *et al.* Antibody-dependent cell-mediated cytotoxicity

induced by active immunotherapy based on racotumomab in non-small cell lung cancer patients. *Cancer Immunology, Immunotherapy*. 67(8), 1285–1296 (2018).
86. Lee L, Wang RF, Wang X, *et al.* NY-ESO-1 may be a potential target for lung cancer immunotherapy. *Cancer J Sci Am*. 5(1), 20–25 (1999).
87. Kim SH, Lee S, Lee CH, *et al.* Expression of Cancer-Testis Antigens MAGE-A3/6 and NY-ESO-1 in Non-Small-Cell Lung Carcinomas and Their Relationship with Immune Cell Infiltration. *Lung*. 187(6), 401–411 (2009).
88. RAFEi H, El-Bahesh E, FiNiANOS A, NASSEREDDiNE S, Tabbara I. Immune-based Therapies for Non-small Cell Lung Cancer. *Anticancer Research*. 37(2), 377–388 (2017).
89. Heery CR, O'Sullivan-Coyne G, Madan RA, *et al.* Avelumab for metastatic or locally advanced previously treated solid tumours (JAVELIN Solid Tumor): a phase 1a, multicohort, dose-escalation trial. *The Lancet Oncology*. 18(5), 587–598 (2017).
90. La Fleur L, Boura VF, Alexeyenko A, *et al.* Expression of scavenger receptor MARCO defines a targetable tumor-associated macrophage subset in non-small cell lung cancer. *International Journal of Cancer*. 143(7), 1741–1752 (2018).
91. Ichiki Y, Hanagiri T, Takenoyama M, *et al.* Tumor specific expression of survivin-2B in lung cancer as a novel target of immunotherapy. *Lung Cancer*. 48(2), 281–289 (2005).
92. Visconti R, Morra F, Guggino G, Celetti A. The between Now and Then of Lung Cancer Chemotherapy and Immunotherapy. *International Journal of Molecular Sciences*. 18(7), 1374 (2017).
93. 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. *Clinical Cancer Research*. 24(7), 1562–1573 (2018).