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Overview of Lung Cancer Immunotherapy

Miguel F. Sanmamed, MD, PhD^{*,†,‡,§}, Iñaki Eguren-Santamaria, MD^{*}, Kurt A. Schalper, MD, PhD^{||}

^{*}Program of Immunology and Immunotherapy, Center for Applied Medical Research

[†]Oncology Department, Clinic University of Navarra

[‡]Navarra Institute for Health Research (IDISNA), Pamplona

[§]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain

^{||}Department of Pathology, Yale University School of Medicine, New Haven, CT

Abstract

Anti-PD-(L)1 therapy represents a turning point in lung cancer immunotherapy, moving from previously ineffective enhancer strategies to immune checkpoints as standard first- and second-line therapies. This unprecedented success highlights the importance of mechanisms to escape immune attack, such PD-1/PD-L1 axis, and emphasize the importance to better understand the tumor immune microenvironment. Analyzing the specifics of immune response against lung tumor cells and how malignant cells progressively adapt to this pressure may help to understand which are the key aspects to guide the development of new therapeutic strategies. Here we review the past and present of clinical lung cancer immunotherapy and give a perspective for the future development based on emerging biological insights.

Keywords

Cancer immunotherapy; lung cancer; tumor immune microenvironment

Immunotherapy strategies based on PD-1/PD-L1 axis blockade have recently demonstrated to be effective in a variety of human malignancies. While the clinical success has been revolutionary, the idea of harnessing the immune system to fight cancer has been tested for over a century. William Coley, in 1890s, experimented introducing bacterial toxins to sarcomas, observing tumor regression in a subset of patients.¹ Since this first proof-of-concept, several immunotherapies have been approved for use in cancer, including interleukin 2 (IL-2), interferon α (IFN- α), Calmette-Guérin bacillus, dendritic-cell vaccine (sipuleucel-T), and anti-cytotoxic T-lymphocyte-associated protein 4 monoclonal antibodies (mAbs). Nevertheless, it was the use of single-agent anti-PD-(L)1 mAbs which have demonstrated for the first time the full potential of immunotherapy in a broad spectrum of tumor types, including those previously considered insensitive to immunotherapy such as lung cancer.² This demonstration has widened the horizon of cancer immunotherapy and

encouraged the development of new approaches to treat most solid and hematological malignancies. Here, we review the past and present of lung cancer immunotherapy and provide a perspective for future treatment strategies based on advances in the understanding of the lung cancer immune microenvironment.

PRINCIPLES OF IMMUNE RESPONSE AGAINST CANCER

Work from the first half of 20th century demonstrated that the immune system has a major role in the control of tumor growth. These studies culminated with the so-called immune surveillance hypothesis of Burnet and Thomas,³ which proposed that our body is constantly under surveillance by our immune system searching for transformed cells to be cleared. Evidence of immune surveillance theory came from both animal models and clinical observations. Mice with a wide variety of immunodeficiencies are more susceptible to transplanted or chemical carcinogen-induced tumors. In the clinic, a spontaneous humoral and cellular immune response against tumor-associated antigens can be detected in most cancer patients, including those with lung cancer. The demonstration of a spontaneous immune response and the identification of specific lung tumor-associated antigens such as NY-ESO-1 or MAGE encouraged the development of different immunotherapy strategies in the 1980s and 1990s. However, the clinical results were disappointing. This discouraging experience, added to the paradox that immunocompetent patients developed cancer and the lack of strong experimental evidence, resulted in reduced interest in immunostimulatory cancer therapies. However, interest was awakened at the beginning of the 20th century when studies from Dunn et al.⁴ and other groups experimentally demonstrated that tumors are able to escape immune surveillance through a process of adaptation generally known as tumor immunoediting. This process was proposed to consist in 3 stages: (1) elimination, (2) equilibrium, and (3) escape. In the latter stage, the tumor evades immune surveillance through the loss of immunogenic antigen presentation and progressive development of an immunosuppressive microenvironment.⁴ This concept has been expanded by the adaptive immune resistance hypothesis, proposed by Lieping Chen, which emphasizes that tumor cells not only adapt to immune pressure by reducing their antigenic potential, but also develop mechanisms to actively suppress the immune attack. This concept was illustrated by the work from Lieping Chen's group in 2002, showing that immunogenic tumors with forced/exogenous expression of PD-L1 (B7-H1) could grow in the presence of an intact immune system.⁵ This seminal work opened new avenues to harness the immune system against cancer, based on the identification and neutralization of dominant tumor immune evasion pathways. This resulted in the clinical development of anti-PD-(L)1 mAbs as the final demonstration that immunotherapy can be effective in a broad spectrum of tumor types including lung cancer.

LUNG CANCER IMMUNOSTIMULATORY AGENTS: FROM ENHANCERS TO IMMUNE ESCAPE TARGETING THERAPIES

Increased understanding of tumor immunology has impacted lung cancer treatment. For over a century, efforts have primarily focused on enhancing immune activation by using cytokines and adjuvant therapies. We have progressively refined our way to harness the antitumor

immune response in a more antigen-specific manner, although clinical results remain limited. It was not until the emergence of anti-PD-(L)1 mAbs when immunotherapy prominently impacted the survival of lung cancer patients opening a new era in the treatment of this disease.

Cytokines and Adjuvants

The first clinical signal for immunotherapy activity in lung cancer came from the administration of high-dose recombinant IL-2 combined with lymphokine-activated killer adoptive therapy in 1985.⁶ Interleukin 2 is a cytokine known for fueling the proliferation of a variety of lymphocyte subsets including those with antitumor antigen specificity. When tested in a phase I clinical trial including different solid tumor types, activity was predominantly observed in renal cell carcinoma and melanoma patients.⁷ This finding led to the development of IL-2⁸⁻¹¹ and subsequent regulatory approval for advanced renal cell carcinoma (1992) and melanoma (1998) by the Food and Drug Administration. In contrast, single-agent IL-2 induced only anecdotal clinical responses in lung cancer patients (3 of 7) and IL-2 combined with chemotherapy reached disappointing clinical results (Table 1).^{12,13} Cytokine therapy was further explored by the use of type I IFNs in the 1990s. Specifically, IFN- α administration improved survival in patients with surgically resected high-risk melanoma and advanced renal cell carcinoma.⁵⁸ IFN- α was also tested in lung malignancies, mainly small cell lung cancer (SCLC), but showed inconsistent impact on survival (Table 1).¹⁴⁻¹⁶ Alternatively, adjuvant therapies have been explored to favor an innate immune response in lung cancer patients through toll-like receptor or dendritic cell maturation agents, but results have also been disappointing (Table 1).¹⁷⁻²⁰

Vaccines

Immunotherapy efforts in lung cancer have also included a variety of strategies to improve effective tumor immune recognition. One of the most prolific has been vaccination, which includes attempts to increase antigen exposure, uptake by antigen-presenting cells, and appropriate T-cell priming. It is broadly accepted that tumor antigens presented on major histocompatibility complex (MHC) molecules to effector T cells are fundamental to mediate an effective adaptive antitumor response.⁵⁹ However, the process to unveil which tumor antigens are being presented by malignant cells and their relative contribution to cancer elimination remains elusive and technically challenging. Although antigen recognition is expected to target mainly aberrant neopeptides modified by nonsynonymous mutations or abnormal posttranslational processes (neoantigens), a variety of other tumor-associated antigens such as primitive germ-cell proteins can be boosted by vaccination strategies. Besides the antigen that is exploited by a vaccine, these therapeutic agents can be classified according to the vehicle used to deliver it to patients, which includes peptides, coding mRNA, and a variety of cellular components involved in the early steps of antigen expression, uptake, and presentation for T-cell priming. In the last decades, multiple vaccination strategies have been developed for lung cancer patients (Table 1).^{21-30,32,34-40} However, modest clinical outcomes, complex manufacturing processes, and the recent advent immune checkpoint blockers have limited their clinical impact. Modern personalized neopeptide-based strategies^{31,33} and combination with checkpoint blockers might reactivate this therapeutic modality in the future.

Bispecific T-Cell Engagers

An innovative antigen-specific therapeutic approach is based on the use of mAbs targeting oncogenic proteins preferentially expressed in cancer cells or bispecific T-cell engagers (BiTEs). These compounds favor the spatial proximity and interaction between tumor and effector immune cells. Cetuximab, an anti-epidermal growth factor receptor (EGFR) mAb extensively used in patients with advanced colorectal cancer, has been studied in lung adenocarcinomas because of the consistent expression of surface EGFR and the preclinical evidence that cetuximab-mediated tumor lysis is enhanced by natural killer (NK) cells.⁶⁰ Bispecific T-cell engagers have become standard clinical practice for treatment of CD19⁺ acute lymphocytic leukemia. In solid tumors, preliminary attempts have been made to target tumor-associated (non-mutated) antigens. However, elevated toxicity rates have confirmed the inherent difficulties of targeting epitopes not restricted to cancer cells. The limited evidence regarding BiTE administration in lung cancer patients is reviewed in Table 1.⁴¹⁻⁵⁰

Adoptive Cell Therapy

Alternative approaches exist to enhance tumor antigen recognition while maintaining restriction by MHC molecules. Adoptive cell therapies (ACTs) harvest autologous tumor-infiltrating lymphocytes (TILs), followed by *ex vivo* culture/expansion/selection and reinfusion to patients with the goal of increasing effector antitumor responses and limit recognition of self-antigens. An increasing variety of culture protocols are being developed to accurately select tumor antigen-reactive effector T cells. Furthermore, a modality of ACT comprises the transfection of a T-cell receptor (TCR) gene with a known specificity for an antigen while maintaining MHC-mediated restriction, known as TCR-transduced T-cell therapy. There is preliminary evidence for this approach in solid tumors, although still anecdotal in lung cancer (Table 1).^{51-54,56,57} There are numerous caveats in TCR-transfected and chimeric antigen receptor (CAR) T-cell ACT development for solid tumors including the common absence of specific target antigens, phenotypic heterogeneity, the presence of dominant immune-suppressive signals in the tumor bed, and the architectural/physical complexity of most solid malignancies. Toxicity is also a concern and is mainly related to the use of preparative chemotherapy regimens and the effect of the therapeutic cell product.⁶¹

Immune-Scape Targeting Drugs: Anti-PD-(L)1 mAbs

In 2015, the anti-PD-1 nivolumab showed a significant overall survival (OS) improvement over docetaxel as second-line treatment for advanced non-small cell lung cancer (NSCLC),^{62,63} which was replicated shortly thereafter by another anti-PD-1 agent pembrolizumab⁶⁴ and the anti-PD-L1 mAb atezolizumab.⁶⁵ The impact of targeting the PD-1/PD-L1 axis in lung cancer was reinforced by the results of the KEYNOTE-024 trial, where pembrolizumab showed superior OS benefit over the first-line platinum-based chemotherapy in patients with PD-L1 protein expression in at least 50% of tumor cells.⁶⁶ Since 2015, it has been a revolution of unprecedented paradigm changes in advanced lung cancer immunotherapy, a shift that has also reached SCLC patients.^{67,68}

Anti-PD-(L)1 success in lung cancer highlights the importance of the PD-1/PD-L1 axis as a dominant tumor immune-inhibitory route and a therapeutic target in cancer immunotherapy.

It is important to acknowledge, nevertheless, that the success of anti-PD-(L)1 agents has not been uniform across different tumor types. Overall response rate has been 2-fold higher in melanoma than in lung cancer. In contrast, only anecdotal activity has been reported in other solid tumors such as prostate, pancreatic, and microsatellite-stable colorectal carcinomas. These differences are unlikely to stem from the immune system configuration of patients, but rather from distinct oncogenesis process and the presence of variably represented dominant immune evasion pathways across malignancies. One practical consequence of this is that the tumor microenvironment may vary significantly across tumor types, demanding a deeper understanding of immune evasion pathways in individual tumors to decide rational and potentially more effective strategies to unlock a “deficient” immune response. The use of generic immunostimulatory treatment in cases with dissimilar immune properties and regulation could at least partially explain the treatment failure because a proportion of malignancies may not rely on the treatment targets for immune evasion. This could be analogous to using targeted therapies in the absence of a specific dominant oncogenic defect. Therefore, a deeper analysis and understanding of the dominant immune evasion pathways and the associated immune-cell defects developed in each tumor will be critical to advance toward a more biologically directed cancer immunotherapy.

TARGETING IMMUNE TUMOR MICROENVIRONMENT

Specific Determinants of the Immune Response in Lung Microenvironment

Immune response against cancer is determined by multiple factors. Some of these factors are tissue-related and may condition a different type of immune response against nascent transformed cells within different organs. The respiratory epithelium, like the gut epithelium, has distinct mechanisms of immune regulation, because of the constant interaction with exogenous/foreign antigens, which could be recognized as “nonself” by the immune system. One of the main differences observed in studies conducted by Farber et al.⁶⁹ and supported by others is that the so-called resident memory T cells are more abundant in these tissues compared with other locations. In this regard, extensive analysis of TILs across different tumor types has shown qualitative and quantitative differences. Dysfunctional regulatory molecules, including transcriptional factors ZBED2 and ID3, were more expressed in melanoma than lung cancer CD8 TILs, suggesting a differential functional modulation of these cells. In addition, T cells with a dysfunctional phenotype were more abundant in melanoma compared with lung cancer samples (median, 11.6% lung cancer vs. 28.9% melanoma).⁷⁰ Another factor likely to participate in the immune response against airway cancers is the lung microbiota. In addition to gut microbiota, the local lung microbiome has been recently characterized.^{71,72} The role of the lung microbiome in maintaining pulmonary homeostasis and its implications in the antitumor immune response and efficacy of immunotherapies are currently under active investigation.⁷³

Transformation From a Normal Immune Ecosystem to a Hostile Lung Tumor Niche

The tumor and immune cell interactions during lung cancer progression have been recently described by Jérôme Galon's group, mapping the local immune changes in low-grade precancerous, high-grade precancerous, and invasive lung tumors.⁷⁴ These studies found that the immune response emerges from a predominantly innate immune reaction during the

earliest steps of transformation, to the appearance of an adaptive T-cell response and associated immune-inhibitory mechanisms in high-grade precancerous lesions. This suggests that the equilibrium between tumor and the immune system is lost at some point between the transition from low- to high-grade precancerous lesions. Similarly, Lavin et al.⁷⁵ evaluated the immune landscape in 28 patients with early-clinical-stage lung cancer compared with paired normal lung. They found that while T cells increased in number in cancer, they showed less granzyme B and IFN- γ production. Different studies comparing tumor and paired nontumor lung tissues have also demonstrated that T helper 1 cell signature and NK cell abundance decrease in cancer. Furthermore, while effector cells decrease in number, 2 immunoregulatory populations, regulatory T cells (Tregs) and neutrophils, increase in the tumor microenvironment,^{75,76} as illustrated in Figure 1. Specific mechanisms driving these changes are not fully determined, but transforming growth factor β (TGF- β) signaling has been linked with increased Tregs, and IL-8 and IL-6 have been described in different studies as 2 cytokines highly expressed by lung cancer microenvironment and implicated in the chemoattraction of immunosuppressive myeloid populations. This immune-suppressive microenvironment could favor the overexpression of additional immune-regulatory systems.

Immune-Evasion Mechanisms in the Lung Tumor Microenvironment

Deficiency of the immune response against cancer in immunocompetent subjects is explained by evasion mechanisms developed by tumors, which may affect the innate and/or adaptive antitumor-immune response. Collectively, immune-evasion mechanisms are considered the different strategies developed by tumors to actively frustrate the immune activation process to recognize and clear tumor cells, going from silencing of chemokines necessary for the attraction of key immune cells to the tumor bed, to the overexpression of immune inhibitory molecules that impair the function of effector immune cells. Among these, the best characterized is the up-regulation of PD-L1/PD-1 pathway, which induce the inhibition of tumor-specific TILs. The success of single-agent anti-PD-1 or anti-PD-L1 supports the importance of this pathway in the lung cancer immunity, because the modulation of a single immune-regulatory axis is able to restore a functional antitumor-immune response in ~20% from unselected NSCLC population. A large proportion of patients, however, are not sensitive to this single-agent modulation, suggesting that other immune-inhibitory mechanisms complementary or alternatively to PD-1/PD-L1 axis are involved. In this regard, studies from our group have assessed the simultaneous expression of 3 immune-inhibitory pathways in lung tumors: PD-L1, IDO-1 and B7-H4.⁷⁷ Interestingly, this work demonstrated that the targets showed a nearly mutually exclusive pattern of expression in tumor cells. This was unexpected considering that expression of both PD-L1 and IDO-1 is experimentally induced by IFN- γ . Notably, the differential expression of these targets in human lung malignancies has also been confirmed by other investigators.⁷⁸ These findings suggest that different mechanisms of immune-inhibition may be present in lung tumors, but only one (or few) of them are selected as a dominant mechanism of adaptive immune resistance at a given time. To date, TCR-ligand immune-inhibitory pathways such as VISTA, B7-H3, TIM-3, TIGIT, and LAG-3 have been observed expressed in lung cancer microenvironment.⁷⁹ The latter is of special relevance because a new soluble activating ligand of this receptor, termed fibrinogen-like protein 1, has been recently observed to be overexpressed in plasma and tumor cells from lung cancer patients displaying poor

prognosis.⁸⁰ Additionally, elevated baseline expression of LAG-3 in lung TILs has been associated with worse survival in patients treated with single-agent anti-PD-1.⁸¹ Collectively, these studies support that LAG-3/fibrinogen-like protein 1 pathway may inhibit tumor-infiltrating T-cell responses independent from the PD-1/PD-L1 pathway and could be used as a therapeutic target in a fraction of NSCLC patients. Other pathways to suppress the antitumor immune response, not related with a ligand-receptor system, have been studied in lung tumors. Recently, it has been shown that lung tumor cells produce IL-8⁸² and that higher tumor IL-8 levels are associated with accumulation of tumor-infiltrating neutrophils/monocytes and worse clinical response to single-agent therapy anti-PD-(L)1.⁸³ The levels of IL-8 were independent from the tumor PD-L1 expression and tumor mutational burden (TMB), supporting an independent effect of this pathway in a fraction of lung carcinomas. The mechanisms associated with the IL-8 effect are not completely elucidated, but they seem to involve CXCR1/2 signaling in neutrophils/myeloid-derived suppressor cells and the local production of neutrophil extracellular traps able to directly suppress effector cells.⁸⁴ Therefore, IL-8 represents a new potential therapeutic target in lung cancer, based in cytokine/cytokine receptor systems. Additional targets in this category include TGF- β and IL-6, both with well-established immune-suppressive activity and therapeutic potential.⁸⁵ Recently, specific glycoproteins have been also identified as a potential mechanism of immune inhibition in lung cancer. The expression of a member of Siglec family, Siglec-15, has been observed in PD-L1-negative tumors.⁸⁶ Interestingly, IFN- γ which induce the expression of PD-L1, inhibits the expression of Siglec-15. Therefore, this could be an interesting alternative immunotherapy for PD-L1-negative NSCLC patients. Nevertheless, fundamental aspects of this new immune target therapy remain unclear including if Siglec-15 inhibits directly the T cells or acts through the modulation of myeloid cells in the tumor.⁸⁷ In a phase I clinical trial using an antagonist Siglec-15 mAb (NC318), one complete response and a partial response have been observed in 2 of 13 lung cancer patients previously treated with anti-PD-(L)1 agents, supporting the relevance of this molecule in lung cancer immunity ([NCT03665285](#)).

LUNG CANCER IMMUNOTHERAPY BASED ON TUMOR IMMUNE MICROENVIRONMENT

Despite the prominent improvement of immunotherapy to treat lung cancer patients, most of responses to anti-PD-(L)1 agents are not maintained and at least 60% of the patients show limited clinical benefit. Combinatorial treatments are perceived as the next frontier to advance lung cancer immunotherapy field, but a strong biological rationale is needed to guide the myriad of potential combinations. Active drugs in NSCLC, such as cisplatin-based chemotherapy regimens or ipilimumab, have been the first drugs successfully combined with anti-PD-1 in NSCLC.^{88,89} However, none of these combinatorial regimens have been truly designed to treat a specific group of lung cancer patients based on their specific biology. PD-L1 and more recently TMB are being used as clinical-grade biomarkers to randomized patients in clinical trials looking for populations with greater benefit to the different therapeutic approaches. A deeper analysis, however, could be used to understand a priori which is the main impairment for the immune system to control tumor growth in each group of patients and select the best treatment strategy accordingly. Different approaches have

been proposed to identify sensitive and resistant patients to anti-PD-(L)1 therapies such as the tumor-immune microenvironment classification by Lieping Chen using T-cell infiltration and PD-L1 expression⁹⁰ or alternatively the combination of TMB and T-cell gene expression profile, recently explored in a large number of cancer patients treated with pembrolizumab.⁹¹ These 2 approaches use TIL abundance as one of the main determinant parameters to define tumors as T-cell inflamed and non-T-cell inflamed. While conceptually this is an interesting approach, it needs to be clinically translated into a specific test to define how many T cells are needed to confer clinical responses to treatment. Unfortunately, methods such as DNA or RNA sequencing using disaggregated/ground tissues may not be able to obtain the cell-level resolution to interpret the immune markers or address the relevant spatial context. Different techniques have been used to determine the presence and abundance of T cells in lung tumors, as some have demonstrated the association between response to checkpoint inhibitors and TIL abundance in NSCLC.⁹² However, the lack of harmonization across studies limits their comparison and the establishment of clinically relevant assays and cutoff points. Furthermore, the classification between T-cell inflamed and non-T-cell inflamed is only the first step in order to define the immune contexture of lung cancer patients. From a biological standpoint, if a tumor is classified as non-T-cell inflamed, the next question that should be addressed is why the T cells are not infiltrating the tumor. Potential causes go from deficient T-cell priming in the lymph nodes, trafficking to the tumor, or tumor tissue infiltration. For instance, STK11/LKB1 mutated lung adenocarcinomas are associated with less T-cell infiltration, and different studies have proposed that this is related with a higher tumor infiltration by myeloid-derived neutrophil-like suppressor cells attracted by chemotactic cytokines IL-6, G-CSF, and CXCL7 highly expressed by STK11-mutated tumors.⁹³ In these tumors, strategies designed to block these cytokines, especially IL-6, may result in a less immunosuppressive environment that allow T-cell infiltration/proliferation and the restoration of an effective antitumor immune function. The identification of actionable causes of T-cell exclusion may define groups of patients who may benefit from specific strategies to overcome this particular deficiency, avoiding overtreatment with other strategies focused on restoring or enhancing TIL function, when these cells are absent. In contrast, in T-cell-inflamed tumors, the question that needs to be answered is why these tumor-infiltrating T cells are not controlling the tumor growth. Here, at least 2 groups can be conceptually defined: (i) patients with “ignorant T cells,” which are present in the tumor but incapable to recognize the tumor cells; and (ii) patients with “overregulated/exhausted T cells,” which are activated upon antigen recognition, but T-cell regulatory mechanisms limit their response (algorithm illustrated as Fig. 2). The first group could be identified by the presence of abundant TILs in the absence of robust effector responses. Causes of this lack of antigen recognition could include epitope silencing, such as B2M loss and/or MHC silencing. Again, the identification of the specific causes may guide the most effective solution going from chemotherapy to expose intracellular antigens, adoptively providing specific T cells (autologous TIL or TCR-transfected T cells), or enhancing the antitumor immune response by other non-T immune cells such as NK cells. The second group, with predominance of overregulated/exhausted T cells, could be identified by the presence of TILs with marked effector responses such as IFN- γ production (Fig. 2). In this case, tumor-specific T cells are potentially recognizing tumor cells and able to infiltrate the tumor bed, but dominant local immune-regulatory mechanisms may be overexpressed. Immune-

regulatory molecules such as PD-1/PD-L1, IDO-1, VISTA, LAG-3, TIM-3, or CD47 have been observed highly expressed in T-cell-inflamed lung tumors. In this case, the challenge stems from the identification of the immune-regulatory pathway predominantly suppressing the T-cell function, especially when more than one of this immune-regulatory mechanism is expressed. PD-1/PD-L1 has shown to be a “master shift” represented in a large proportion of NSCLCs, and its blockade is able to reset the immune dynamics, but this may not be the case for all the immune-inhibitory mechanisms, and identifying their hierarchy in a certain patient is one of the biggest challenges toward a more personalized immunotherapy. New analysis tools and advanced preclinical models able to recapitulate this complexity are needed to address these questions in order to better define which are the “driver” immune-regulatory pathways and which can be considered as “passengers.” The next generation of clinical trials in lung cancer immunotherapy could integrate these expanding understanding of the immune tumor microenvironment and apply it in a more tailored manner by targeting different mechanisms of adaptive immune resistance. In this regard, the validation of biomarkers to classify tumors based in the presence of T-cell infiltration or T-cell activation will be required to better select strategies to enhance an effective antitumor immune response for each patient (Fig. 2).

CONCLUSIONS

After decades of immunotherapy failures, targeting the immune tumor microenvironment with PD-1/PD-L1 axis blockers has shown to significantly improve the survival in a fraction of lung cancer patients, opening a new way to treat this disease. Immune-escape mechanisms developed by each tumor may be different, as each one occurs in different organs with variable tissue homeostasis determinants. A better understanding of these determinants and the specific mechanisms developed by lung tumor cells to avoid, or overcome, immune attack are of paramount importance to advance the field. Progress in the analysis of the specific antitumor-immune deficiencies may guide the development of new lung cancer immunotherapies and more rational treatment combinations.

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FIGURE 1.

Immune microenvironment of nontumor lung tissue and lung cancer. Schema of the immune cell composition and immunomodulatory determinants in nontumor lung (left) and lung cancer (right). The nontumoral lung is characterized by a dynamic homeostatic balance between proinflammatory and immune-suppressive signals allowing continuous immune surveillance and preventing tissue/organ damage. The airway microbiota and allergens may have a role in shaping the local immunity. The nontumoral lung contains a large proportion of resident effector memory T cells compared with other tissues with less antigenic exposure. The tumor microenvironment is characterized by unbalanced immune activating and regulatory signals. Lung tumors display expansion of T-cell subsets and myeloid populations including neutrophils and tumor-associated macrophages (TAMs). In contrast, NK cells are typically decreased. Immune-suppressive cytokines upregulated by malignant cells include TGF- β , GM-CSF, IL-8, and IL-6 (black arrows). They promote the recruitment, differentiation, or expansion of Tregs and myeloid-derived suppressor cells. Immune inhibitory ligands, cytokines, and metabolites overexpressed in lung tumors can directly regulate the function of effector T cells (red arrows). Figure 1 created with [BioRender.com](https://www.biorender.com).

FIGURE 2.

Potential for developing anticancer immunotherapy strategies based on the dominant immune evasion pathways. Immune response against tumor cells may fail because of impairments at multiple levels of the immune activation process. Validated biomarkers could be used to define the immunological lung cancer subsets based on T-cell responses and pinpoint the biologically relevant alterations. Conceptually, 3 salient groups could be defined using this approach: T-cell exclusion, T-cell ignorance, and T-cell overregulation/exhaustion. Enhanced biomarkers will be required to identify these groups using functional T-cell markers and understand the underlying causes. Each T-cell subset is likely to be associated with specific tumor and immune-cell defects, and this information could be used to decide more tailored therapeutic interventions. In the figure, the potential groups of causes that can determine each impairment state and biologically oriented strategies to overcome it are depicted.

Table 1.

Previous experience in lung cancer immunotherapy

CYTOKINES				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
IL-2	Rosenberg 1994 Rosenberg 1998 Dillman 1993 Fyfe 1996 Klapper 2008	14-20% RR in patients in melanoma and RCC patients. 5-9% CRR, majority of them long-lasting. FDA-approved for RCC in 1992. FDA-approved for melanoma in 1998.	Limited experience in lung cancer patients (3/7 responders). Severe, commonly transient, toxicity due to increased vascular permeability. Need for intensive monitoring and vasoactive drugs.	
IL-2 combinations	Lissoni 1994	Phase 2 trial comparing low-dose IL-2 + melatonin vs. CDDP + VP-16 in NSCLC; similar RR, PFS and OS in the chemotherapy-free group.		
	Ridolfi 2011	Phase 3 trial comparing CT +/- low dose IL-2 in NSCLC; no relevant differences in clinical outcomes.	Higher G4 toxicity in the IL-2 containing arm.	
IFNα	Prior 1997 Ruotsalainen 1999 Tummarillo 1997	IFN α -2b FDA-approved in 1995 for adjuvant treatment of high-risk resected melanoma. Three randomized placebo- controlled phase 3 trials in SCLC: CT +/- IFN α . Improved RR and OS have been reported in some (Prior, Tummarillo), while others failed to show influence on survival (Ruotsalainen).	Pyrexia and grade 3-4 hematologic AEs were more frequent in the IFN α arms.	
INNATE IMMUNITY AND ADJUVANTS				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
TLR-9 agonist: PF-3512676	Manegold 2008	Randomized placebo controlled phase 2 trial for advanced CT-naïve NSCLC patients: CT +/- PF-3512676. Improved response rate: 19 vs. 11%. Trend towards prolonged OS.	Grade 3 anemia, thrombocytopenia and neutropenia more commonly reported in the PF-3512676 arm.	
Bacterial adjuvants Killed <i>Mycobacterium vaccae</i>	O'Brien 2004	Randomized phase 3 trial in advanced NSCLC: CT +/- intradermal vaccination. No difference in OS.	No relevant safety concerns.	
Bacterial adjuvants Killed <i>Mycobacterium indicus pranii</i>	Belani 2017	Randomized placebo-controlled trial for treatment naïve advanced NSCLC: CT +/- vaccine. No OS differences in the ITT population. Subgroup analyses suggested SCC patients derived a significant OS benefit.	No differences in systemic AEs.	
DC maturation agents Talactoferrin	Ramalingam 2013	Placebo-controlled phase 3 trial.	No differences in DCR, PFS or OS.	
VACCINES				
Peptide-based antigen delivery				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
EGF conjugated with N. meningitidis P64K peptide (CY 200 mg/m ² before first vaccine dose)	Neninger-Vinageras 2008	Randomized phase 2 trial of maintenance therapy after first-line CT for advanced NSCLC. Statistically non-significant trend towards prolonged OS.	No grade 3-4 AEs observed.	

CYTOKINES				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
	Xing 2018	Phase 1, single arm vaccination trial for patients with advanced NSCLC who remain PD-free after first-line CT. No objective responses reported.	No DLTs in the four dose levels explored.	
MUC1 (EMA)-derived peptide in a liposomal preparation (CY 300 mg/m ² before first vaccine dose)	Butts 2005 Butts 2011 Butts 2014 Katakami 2017	Expressed in most ADCs. Randomized phase 2b trial of maintenance therapy after first-line CT for advanced NSCLC. Non-significant trend towards prolonged OS. OS benefit is greater in patients with locally advanced disease (subgroup analysis) (Butts, 2005 and 2011). Randomized phase 3 placebo-controlled maintenance therapy after CRT for stage III NSCLC. Non-significant trend toward improved OS. Greater benefit in patients who had received concurrent vs. sequential CRT (subgroup analysis) (Butts 2014). This was not confirmed in a phase 2 trial conducted in Japanese patients with stage III NSCLC (Katakami).	No relevant safety concerns. Slightly higher incidence of CNS metastases in the vaccination arm in the phase 3 trial.	
GV-1001: telomerase reverse transcriptase (hTERT) peptide + GM-CSF 75 µg	Brunsvig 2011	Phase 1 trial in advanced NSCLC patients. Significantly prolonged OS in patients that had an in vitro T-cell response to the peptide. Phase 2 trial of vaccination after CRT for stage III NSCLC. Trend toward improved PFS in patients that had an in vitro T-cell response to the peptide.	No serious TRAEs.	
IDO-peptide	Kjeldsen 2018	Phase 1 single-arm trial for advanced NSCLC patients who remained PD-free after CT. 2/15 objective responses. 6-year OS: 3/15.	No grade 3-4 AEs in long-term responders (2/15).	
Peptide vaccination according to baseline IgG titers against five antigens	Sakamoto 2017	Phase 2 trial in advanced SCLC patients. IgG responses to non-vaccinated peptides were associated with OS.	Grade-3 AEs were limited to the skin.	
Racotumomab: anti-NeuGcGM3 ganglioside anti-idiotype antibody	Alfonso 2014	Phase 2/3 placebo-controlled maintenance therapy for advanced NSCLC patients after first-line therapy. Statistically significant prolongation of PFS and OS. Neutralizing and cytotoxic IgM seroconversion was associated with survival.	No relevant safety concerns.	
Peptide-based neo-epitope vaccination	Keskin 2018	Induction of neo-epitope specific T cell responses in glioblastoma patients.	Limited experience in lung cancer	
mRNA-based antigen delivery				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
mRNA encoding for five tumor-associated antigens: NY-ESO-1, MAGE-C1, MAGE-C2, surviving and 5T4	Sebastian 2019	Phase 1/2a single-arm trial for advanced NSCLC patients who remained PD-free after first-line treatment. No objective responses. Median PFS: 5.0 m. Median OS: 10.8 m.	No grade 4 TRAEs.	
RNA-based poly-neo-epitope vaccination	Sahin 2017	Induction of neo-epitope specific T cell responses in melanoma patients. 2/5 stage-IV melanoma responders.	Limited experience in lung cancer.	
Cell-based antigen delivery				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	

CYTOKINES				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
Autologous GM-CSF-producing irradiated tumor cells	Davies 2007	Phase II single-arm trial in patients with advanced BAC. No objective responses.	Patients have to undergo surgery to obtain tumor tissue. Complex manufacturing process that led to low accrual.	
Allogeneic intradermal tumor-cell lines transfected with TGF- β 2 antisense vector.	Giaccone 2015	Phase 3 randomized placebo-controlled maintenance therapy after front-line therapy for advanced NSCLC. No OS benefit in the ITT analysis. Positive OS prolongation in patients who received CRT prior to randomization (pre-specified subgroup analysis).	No serious safety concerns.	
Tergenpumatucl-L: allogeneic intradermal tumor-cell lines modified to express α 1,3-Gal	Morris 2013	Phase 2 single-arm trial for previously-treated advanced NSCLC. 8/28 SD lasting 16 weeks. Median OS: 11.3 months. 9/16 responses to subsequent CT, suggesting a chemo-sensitization effect.	No concerning safety issues.	
GM-CSF- and CD40L-producing bystander cell line + two allogeneic tumor cell lines +/- CCL21-producing adenoviral vector	Gray 2018	Phase 1/2 trial for previously treated ADC patients. No PFS or OS differences between treatment groups.	No grade 3 toxicity reported.	
Viral vector-based antigen delivery				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
Lentivirus-NY-ESO-1	Somaiah 2019	Anti-NY-ESO-1-specific CD4 and/or CD8 T cells induced in 57% of patients. DCR: 56% in a cohort composed mainly of patients with sarcomas, melanoma and ovarian cancer. Only 1/38 responder.	Limited clinical evidence in lung cancer (n=1). There might have been central tolerance to eliminate non-mutant protein-reactive T cells.	
TG4010; modified <i>vaccinia Ankara</i> that codes for MUC1 and IL-2	Quoix 2016	Phase 2b placebo-controlled trial for MUC1-expressing (50% tumor-cells by IHC): CT +/- subcutaneous TG4010. Improved RR (40 vs. 29%) and PFS, but not OS in the ITT population. Subgroup analyses suggested that non-squamous tumors and low blood CD16 ⁺ CD56 ⁺ CD69 ⁺ cell counts derived the greatest benefit.	No relevant safety concerns.	
APC delivery				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
Ad.p53-DC; TP53-transfected dendritic cells +/- ATRA	Chiappori 2019	Phase 2 trial of Ad.p53-DC +/- ATRA or placebo in ED-SCLC who remained PD-free after first-line CT, followed by paclitaxel at PD. ORR to paclitaxel was higher in Ad.p53-DC+ATRA compared to Ad.p53-DC monotherapy and placebo arms: 24, 17 and 15% respectively. No significant OS differences, although numerically higher in the placebo arm.	No grade-4 toxicity reported.	
MONOCLONAL ANTIBODIES AND BITES				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
ADCC - Cetuximab	Lynch 2010 Pirker 2009 Rosell 2008	Predictive early-onset acneiform rash may be immune mediated.	Modest OS improvement over standard CT alone. No regulatory agency approval.	
BITEs CEA-CD3	Ordoñez 2006 Tabernero 2017	Positive in a significant proportion of lung ADC and non-keratinizing SCC. 5% RR in CRC (higher if combined with Atezolizumab: 20%).	Limited experience in lung cancer patients.	

CYTOKINES				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
			Significant systemic and on-target (off and on-tumor) toxicity.	
BITEs EpCAM-CD3	Kebenko 2018	Expressed in ADC and SCLC. Nine lung cancer patients included.	Limited activity. Limiting on-target off-tumor toxicity (diarrhea, liver enzymes): 95% of patients with grade 3 AEs.	
BITEs DLL3-CD3	Tanaka 2018 Rudin 2017 Smit 2019 Hipp 2020	32-67% of SCLC with 50% tumor-cell expression. Well tolerated in cynomolgus monkeys.	Only preclinical evidence of activity. Ongoing clinical trials.	
ACT				
MODALITY	REFERENCE	OUTCOMES	CAVEATS	
Anti-NY-ESO-1-TCR-transduced T-cells	Jungbluth 2001 Robbins 2015	Expressed (IHC) in 25% of NSCLC. No relevant off-tumor toxicity (1 death due to preparative CT-induced neutropenia).	Limited clinical evidence in tumors other than melanoma and synovial sarcoma.	
Anti-MAGE TCR-transduced TILs	Kerkar 2016 Morgan 2013 Lu 2017	Expressed in 24-34% lung tumors. 5/9 PR in melanoma and synovial sarcoma (Morgan). 4/17 RR in patients with a variety of solid tumors (Lu).	HLA-restricted. On-target off-tumor neurologic toxicity, higher for MHC-I restricted TCRs. Limited evidence in lung cancer patients.	
PBMC-derived <i>ex vivo</i> stimulated cells	Wu 2008	CT +/- ACT in advanced NSCLC patients. Statistically significant PFS and OS prolongation.	No relevant safety issues reported.	
EGFR-directed CAR T-cells	Feng 2016	Phase-1 single-arm trial for relapsed/refractory NSCLC patients with IHC EGFR expression 50%. RR: 2/11, short lasting responses.	A single grade-3 AE was described: lipase elevation.	

ACT: adoptive cell therapy; ADC: adenocarcinoma; AE(s): adverse event(s); ADCC: antibody-dependent cellular cytotoxicity; APC: antigen-presenting cell; ATRA: all-trans retinoic acid; BAC: bronchoalveolar carcinoma (obsolete term); BiTE: bi-specific T-cell engager; CDDP: Cisplatin; CNS: central nervous system; CRC: colorectal cancer; CRR: complete response rate; CRT: chemoradiotherapy; CT: chemotherapy; DCR: disease control rate; DLT: dose limiting toxicity; ED: extensive-disease; FDA: Food and Drug Administration; G: adverse-event grade; IDO: indoleamine 2,3-dioxygenase; IHC: immunohistochemistry; ITT: intention-to-treat; NSCLC: non-small cell lung cancer; OS: overall survival; PD: progressive disease; PFS: progression-free survival; PR: partial response; RCC: renal cell carcinoma; RR: response rate; SCC: squamous-cell carcinoma; SCLC: small cell lung cancer; SD: stable disease; TLR: toll-like receptor; TRAE: treatment-related adverse event; VP-16: etoposide;