Liquid biopsy for lung cancer immunotherapy (Review)

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Abstract. The recent successful use of the immune checkpoint inhibitors (CPIs) anti-programmed death receptor-1 (PD-1)/PD-1 ligand 1 in clinical trials indicates their crucial role in obtaining an effective cancer immune therapy. These CPIs have been identified to have an effective therapeutic response, particularly in tumors with high tumor mutation burden. Targeting private somatic mutations encoding immunogenic neoantigens (neo-Ags) has been developed as an autologous gene therapy. T-cell receptor-engineered T cells targeting neo-Ags are a novel option for adoptive cell therapy used for the treatment of lung cancer. However, not all patients experience an effective response from immunotherapy. Although the resistance mechanism of CPIs has been reported, its association with other treatment methods during systemic anticancer therapy remains unclear, particularly the treatment options following the emergence of drug resistance in lung cancer. The potential biomarkers used for liquid biopsy may assist in the identification of patients who would benefit the most from immunotherapy. Attempts to identify potential biomarkers for predicting clinical response to immunotherapy are underway. With regard to liquid biopsy, the present review summarizes and discusses the lung cancer management of immunotherapy for precision medicine by reviewing recent literature and associated clinical trials.

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Abbreviations: TCR, T-cell receptor; CPI, checkpoint inhibitor; NSCLC, non-small cell lung cancer; TMB, tumor mutation burden; CTLA4, cytotoxic T-lymphocyte-associated protein 4; TME, tumor microenvironment; GZM, granzyme; neo-Ag, neoantigen; MHC, major histocompatibility complex; TIL, tumor-infiltrating lymphocyte; NGS, next-generation sequencing; HPLC-MS, high-performance liquid chromatography-mass spectrometry

Key words: checkpoint inhibitors, tumor mutation burden, liquid biopsy, T cells, prognosis, lung cancer, neoantigens

Contents

1. Introduction

Checkpoint inhibitors (CPIs), adoptive cell transfer and administration of the cytokine interleukin 2, have been developed as effective clinical cancer immunotherapies, with no clear identification of the immunogenic targets in human types of cancer. Since ipilimumab, an immune CPI for CTLA4 was approved in the United States in 2011 (1), CPIs, as novel anticancer agents, have indicated great promise for effective lung cancer therapy (2-7). Among them, the programmed death receptor-1 (PD-1)/PD-1 ligand 1 (PD-L1) pathway is a key immune checkpoint (8). Anti-PD-1 monoclonal antibodies have been approved by the Food and Drug Administration in the USA for treatments of a number of solid cancer types, including advanced non-small cell lung cancer (NSCLC) (9-15). In addition, antibodies against PD-L1 have indicated an effective clinical response in patients with NSCLC (16).

Spigel *et al* reported an association of tumor mutation burden (TMB) with the effect of CPI therapy (17). Anagnostou *et al* (18) have depicted the evolving landscape of tumor neoantigens (neo-Ags) and immunogenic products of somatic mutations in patients with NSCLC, who exhibit resistance following initial response to CPIs with anti-PD-1 or anti-PD-1/anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) antibodies. This study provided insights into the dynamics of mutational landscapes during CPI therapy and discusses implications for the development of immunotherapies that target private tumor neo-Ags. Increasing clinical evidence has indicated that neo-Ags will become the targets associated with successful immunotherapy.

Liquid biopsy was successful for its utility in molecularly targeted therapy (19-21). Unlike surgical biopsies, it is simple and non-invasive, allowing, through a simple blood sample, an extensive amount of information to be obtained about

the tumor. Clonal evolution with driver gene mutations (e.g., EGFR, EML4-ALK) has allowed lung cancer to become suitable for liquid biopsy in molecularly target therapy (22). With the use of CPIs, ~30% of patients with lung cancer, whose tumor PD-L1 expression is >50% (23), might benefit from better prognosis. However, at the European Society for Medical Oncology 2016 congress, the results indicated that molecularly targeted drugs are available only for subgroups of patients with cancer, and that CPIs are effective in 20-30% of patients, who have not been indicated to have any of the available predictive markers, including PD-L1 and PD-1 (24). However, useful biomarkers that can facilitate the monitoring of lung cancer immunotherapy, particularly liquid biopsy biomarkers, are still lacking (25). In the present review, the immune CPI response/resistance and the change in clinical therapy strategy based on the cancer-immunity cycle, the liquid biopsy biomarkers for lung cancer immunotherapy and a T-cell receptor (TCR)-engineered adoptive therapy targeting neo-Ags was conducted for patients with lung cancer by using liquid biopsy material-circulating tumor cells or circulating tumor DNA (ctDNA) are discussed. The current literature and clinical trials were highlighted regarding the use of liquid biopsies in lung cancer immunotherapy.

2. Cancer-immunity cycle and immune CPI response and resistance

The clinical trials and utility of CPIs have provided key insights into the potential mechanisms of anticancer immune therapies that may underlie cancer immune escape (26). A seven-step event in an anticancer immune response, known as the cancer-immunity cycle (27), is required to be initiated and to sequentially lead to the effective killing of cancer cells. In the last step, the dead cancer cells will release further tumor-associated antigens and cycle again to increase the strength of the immune response in subsequent cycle revolutions. However, the cancer-immunity cycle does not function as aforementioned in patients with cancer. The anticancer function of effector T cells may not respond properly, owing to the factors in the tumor microenvironment (TME) (28) as indicated in Fig. 1A. At the early stage, the tumor possesses a lower TMB/fewer neo-Ags (29). Subsequently, the tumor appears to induce a greater TMB/more neo-Ags through the loss of mismatch repair and DNA instability, enhancing the immunity of cancer, and ultimately leading to activation of tumor neo-Ag-specific cluster of differentiation (CD)8$^+$ T cells and immune-mediated tumor cell death (30-33). Heterogeneity, relevance of neo-Ag burden and importance of clonal vs. subclonal neo-Ag in patients with early-stage NSCLC, included in The Cancer Genome Atlas project, have been assessed (34). Generally, the human body has an immunoregulatory mechanism, known as immune checkpoint, including CTLA4 and PD-1/PD-L1 (35). Using this pathway, the tumor evades the lethal effects of the immune system, therefore neo-Ags, including driver/passenger, serve an important role in this progress. Inhibiting the immune checkpoint and killing the clonal or subclonal neo-Ag-specific tumor cells are useful ways to unlock the suppressed lethal response to tumors (36). Monoclonal antibodies against the PD-1/PD-L1 pathway (Table I), have been proved to improve outcomes in patients with NSCLC (6,11-13,37), including patients who have relapsed following treatment with platinum-based first-line chemotherapy or tyrosine kinase inhibitor therapy (Fig. 1B). The clinical trial of nivolumab in resectable NSCLC (trial no. NCT02259621), investigates the safety, feasibility and effects of nivolumab in this patient population (38). Neoadjuvant nivolumab was indicated to have fewer side effects, to not require any delay in surgery and to induce a major pathological response in 45% of resected tumors (38). Combining immunotherapies with chemotherapy or radiotherapy may lead to an improved response in various patients. One reason for this observation was that cytotoxic chemotherapies and radiation may induce a greater number of novel subclonal mutations that are associated with the response to immunotherapy (39). Furthermore, the immune TME (iTME) will be evaluated or assessed to a greater extent by immune signature/immunogenomic analysis, including quantification of infiltrated CD8$^+$ T cells, though immunohistochemistry (IHC) assay, and their TCR analysis, including next-generation sequencing (NGS) (40,41).

3. Biomarkers associated with liquid biopsy for lung cancer immunotherapy

Currently, liquid biopsy, in particular ctDNA, can indicate better tumor heterogeneity at a greater accuracy compared with tumor biopsy, since it facilitates a convenient and dynamic analysis (42,43). The question is how liquid biopsies can be utilized for immunotherapy. As for immunotherapy, liquid biopsies may be useful for monitoring ctDNA and the response of the immune system *in vivo*, for example, the analysis of circulating free DNA (cfDNA) released from distinct T cell clones, on the basis of the assessment of B cell receptor and TCR immune repertoire from blood plasma (44). The dynamic variation in the cfDNA (45) or T cell-surface markers in the blood (46) may provide clues to the type of treatments that have a higher probability to be effective for each patient. Further study of TME is required, in order to identify suitable biomarkers for liquid biopsy, in particular the iTME. Histologically, the primary tumors can be broadly categorized into two classes: Inflamed or uninflamed (26,47,48). A subset of immune-associated genes, including CD8α/β, interferon (IFN)-γ and granzyme (GZM) A, B and H that were upregulated in the high clonal neo-Ag group, was revealed by gene expression analysis, indicating an inflammatory TME (49). The expression of these genes was countered by the upregulation of immune checkpoints, including PD-1, PD-L1 and PD-L2. The immune CPIs indicated a high efficacy against inflamed tumors, owing to their sufficient infiltration by cytotoxic T cells that recognize cancer-specific antigens or neo-Ags, high density of IFN-γ-producing CD8$^+$ T cells, expression of PD-L1 in tumor-infiltrating immune cells, possible genomic instability, and the presence of a pre-existing antitumor immune response (50). However, they have not been indicated to be effective against uninflamed tumors, which are immunologically unknown, are poorly infiltrated by lymphocytes, rarely express PD-L1, and are characterized by highly proliferating tumors with low TMB and low expression of antigen-presentation machinery markers, including major histocompatibility complex (MHC) class I (51-53).

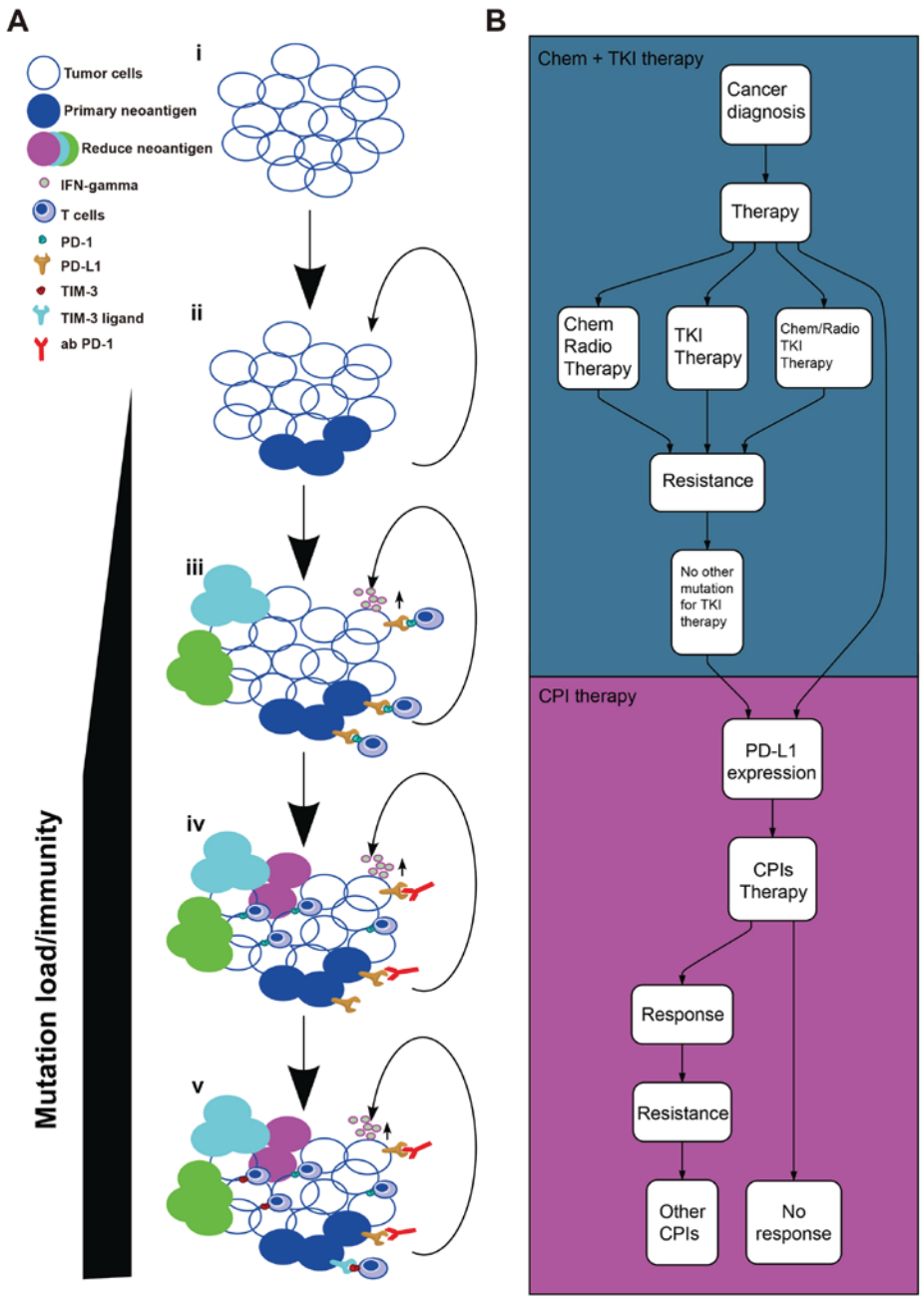

Figure 1. Cancer-immunity cycle and immune checkpoint inhibitor resistance. (A) CPI resistance on the cancer-immunity cycle and TMB/neo-Ags. i) Early-stage cancer without/with fewer neo-Ags. ii) Cancer within major neo-Ags. iii) For resistance of immune response for major neo-Ag, cancer induces an increase in neo-Ags to render cancer less susceptible to the immune system. The CPIs will give a clinical response at this stage. iv) The CPIs, including anti-PD-1, are used. v) Adaptive resistance with upregulation of alternative immune checkpoints-TIM-3. The circular arrow indicates the cancer-immunity cycle. (B) Clinical therapy strategy using chemo-, TKI and CPI. CPI, checkpoint inhibitor; neo-Ag, neoantigen; TMB, tumor mutation burden; TKI, tyrosine kinase inhibitor; PD-1, programmed death receptor-1; PD-L1, PD-1 ligand 1; chem, chemotherapy; radio, radiotherapy; TIM-3, T cell immunoglobulin-3; IFN-γ, interferon γ; ab, antibody.

According to a useful pragmatic framework reported by Teng *et al* (54) and Smyth *et al* (55), TME can be stratified into four types: Type I [tumor-infiltrating lymphocyte (TIL)+, PD-L1+], Type II (TILs-, PD-L1-), Type III (TIL-, PD-L1+) and Type IV (TIL+, PD-L1-) (Fig. 2A). Researchers have attempted to use this classification for lung cancer immunotherapy, in order to provide an explanation for its contribution of its poor prognosis (56-58). Biomarkers associated with distinguishing the four types of iTME will be beneficial to clinical cancer management of individualized and precise cancer treatment.

According to the use of CTLA4, PD-L1 and PD-1, co-inhibitory receptor targets, including lymphocyte activating 3 (59), T cell immunoglobulin-3 (TIM-3) (60) and T cell immunoglobulin and ITIM domain (61), which are safer and less toxic (62), are being investigated in clinical trials. Adaptive resistance to anti-PD-1 therapy is associated with the upregulation of TIM-3 expression in lung cancer (63). Patients with cancer may receive more optimal effects when receiving the anti-TIM-3 agent. The expression level of PD-L2, GZMA and human leukocyte antigens A has indicated that these factors are novel potential biomarkers for predicting the

Table I. PD-1/PD-L1 pathway checkpoint inhibitors FDA-approved or in clinical trials for the treatment of lung cancer.

Target	Agent	Class	Company	Indications (date of approval or publication)	(Refs.)
PD-1	Pembrolizumab (MK-3475)	IgG4	Merck Sharp & Dohme (UK)	First-line treatment if the lung cancer has spread (advanced NSCLC) and tests positive for PD-L1 and tumor exhibits no mutations in EGFR or in ALK; second-line treatment if chemotherapy that contains platinum has already been administered and was unsuccessful or is no longer working. In addition, in lung cancer that test positive for PD-L1, used with chemotherapy medicines pemetrexed and carboplatin, as the first treatment for advanced NSCLC when the type of lung cancer is non-squamous (2017, FDA approved)	(13,96)
	Nivolumab (BMS-936558)	IgG4	Bristol-Myers Squibb (USA)	The treatment of progressive metastatic NSCLC or following platinum-based chemotherapy; patients with EGFR or ALK genomic tumor aberrations should have disease progression FDA-approved therapy prior to receiving nivolumab (2014, FDA approved)	(97,98)
PD-L1	Durvalumab (MEDI4736)	IgG4	Medimmune (USA)	Patients with unresectable stage III NSCLC with no disease progression following concurrent platinum-based chemotherapy and radiation therapy (2017, FDA approved)	(99)
	Atezolizumab (MPDL-3280A)	IgG1	Genentech (USA)	Patients with metastatic NSCLC, whose disease progressed during or following platinum-based chemotherapy; disease progressed during the FDA-approved therapy with EGFR or ALK genomic tumor aberrations prior to receiving atezolizumab (2018, FDA approved)	(86)

PD-1, programmed death receptor-1; PD-L1, PD-1 ligand 1; NSCLC, Non-small cell lung cancer; EGFR, epidermal growth factor receptor; ALK, ALK receptor tyrosine kinase; FDA, Food and Drug Administration; IgG, immunoglobulin G.

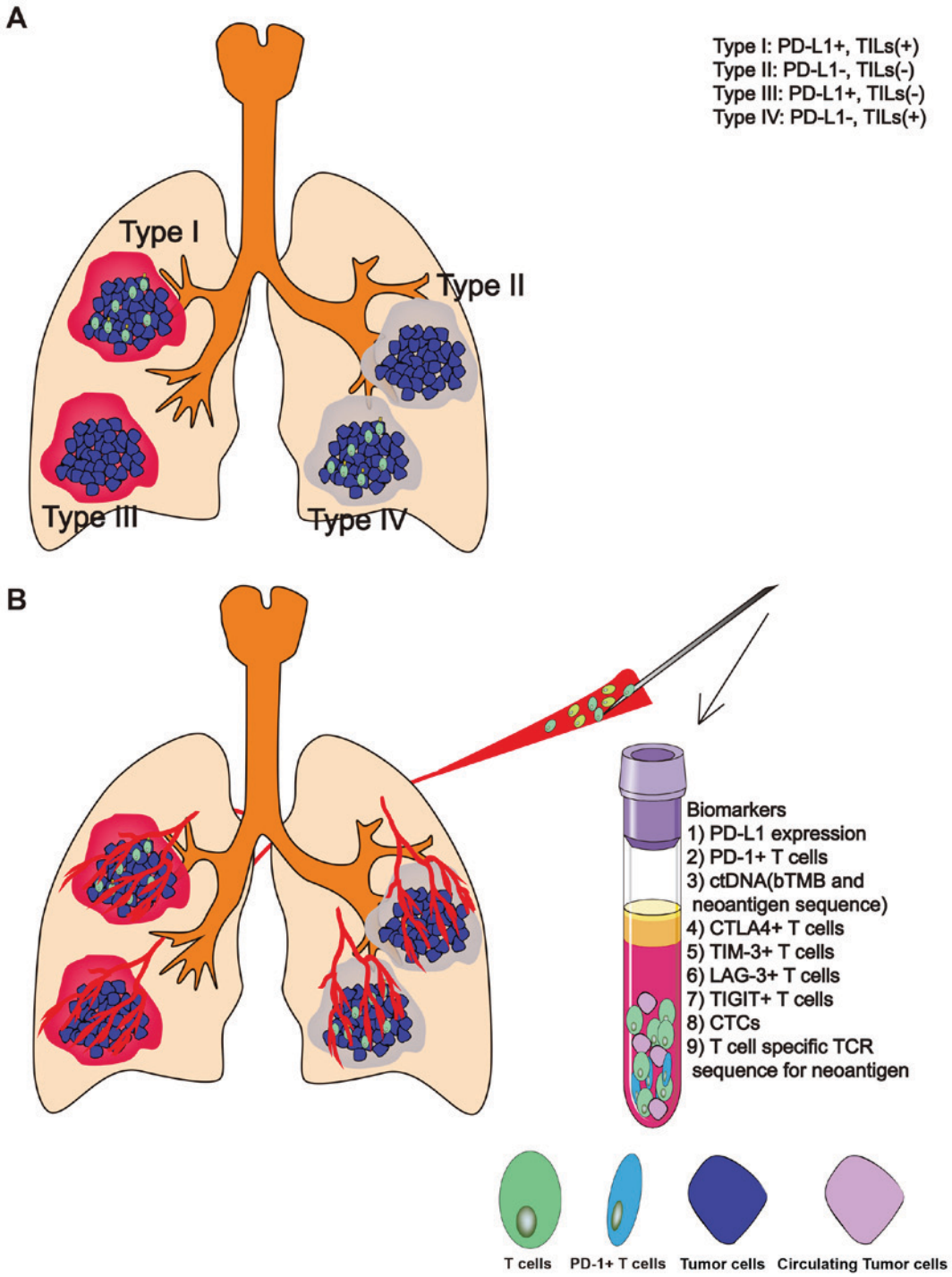

Figure 2. Liquid biopsy for cancer immunotherapy based on PD-1 checkpoint inhibitor in lung cancer. (A) The four types of lung cancer, according to the immune status of tumor microenvironment: Type I, PD-L1+ with TIL+, indicating adaptive immune resistance; Type II, PD-L1- with TIL-, indicating immune ignorance; Type III, PD-L1+ with TIL-, indicating intrinsic induction in iTME; Type IV, PD-L1- with TIL+, indicating the role of other suppressor pathways in promoting immune tolerance. (B) Biomarkers associated with iTME in blood samples. With liquid biopsy by blood sample, the PD-L1 expression, including soluble PD-L1 and cell-free PD-L1 RNA, and surface biomarker expression (i.e., CTLA4, PD-1, LAG-3, TIM-3) on circulating T cells, provide a window into the antitumor reactivity of T cells in the TME. CTCs, ctDNA and cfDNA were used for the detection of TMB or bTMB. TIL, tumor-infiltrating lymphocyte; PD-1, programmed death receptor-1; TIM-3, hepatitis A virus cellular receptor 2; iTME, immune tumor microenvironment; CTLA4, cytotoxic T-lymphocyte-associated protein 4; CTCs, positive circulating tumor cells; ctDNA, circulating tumor DNA; cfDNA, circulating free DNA; TMB, tumor mutation burden; TCR, T-cell receptor; LAG-3, lymphocyte activating 3.

effective response for CPIs in pre-anti-PD-1 antibody-treatment (nivolumab) melanoma tissues (50). Gros *et al* (64) reported that mutation-specific T cells may be isolated from blood in 75% of patients with melanoma. This study was focused on melanoma; however, it is becoming clear that immunotherapies can be used to treat numerous types of cancer, including lung cancer. These mutation-specific T cells have made it possible to determine the neo-Ag status of tumors from blood, and they may serve as a liquid biopsy technique for cancer immunotherapy or a novel immunotherapy (41). In Fig. 2B, the biomarkers associated with

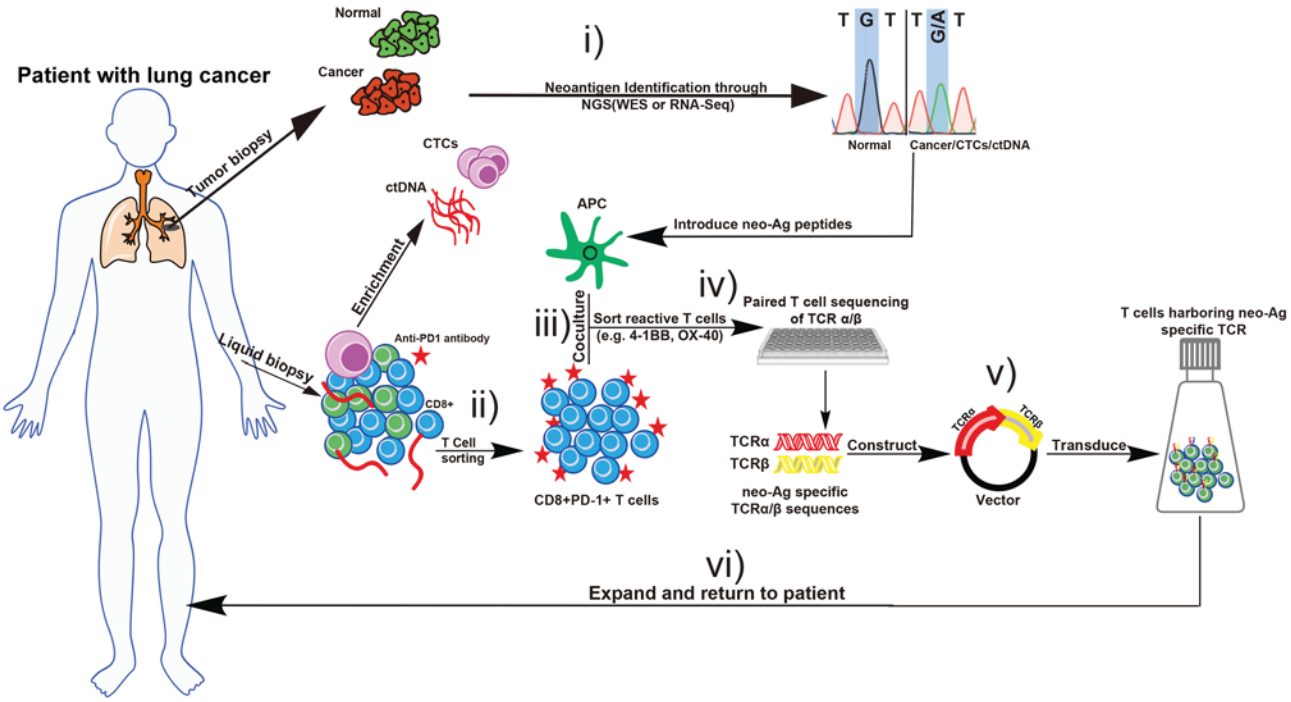

Figure 3. TCR-engineered adoptive therapy targeting neoantigen in patients with lung cancer. i) Primary tumor biopsy or CTCs/ctDNA enriched from liquid biopsy, underwent whole-exome sequencing and RNA sequencing to identify non-synonymous somatic mutations or neo-Ags. ii) CD8+/PD-1+ T cells were sorted by flow cytometry. iii) Sorted CD8+/PD-1+ T cells were co-cultured with antigen-presenting cells with synthetic long peptides of neo-Ag. iv) T cells with upregulated activation markers, including 4-1BB, OX-40, were isolated and underwent paired TCR sequencing to identify TCRα/β sequences against neo-Ag. v) T cells isolated from the blood cells of the same patient were modified with the transfect vector to encode the identified TCRα/β. In this process, the patients' T cells acquired tumor-specificity that allowed them to attack cancer with specific neo-Ag. vi) Modified T cells were cultured and expanded *in vitro* to obtain sufficient numbers for the treatment and reinfusion into the same patient with cancer. TCR, T-cell receptor; CTCs, positive circulating tumor cells; ctDNA, circulating tumor DNA; neo-Ags, neoantigens; PD-1, programmed death receptor-1; NGS, next-generation sequencing; WES, whole exome sequencing; OX-40, tumor necrosis factor superfamily member 4; 4-1BB, tumor necrosis factor receptor superfamily member 9; CD, cluster of differentiation.

iTME are depicted, which may be used in liquid biopsy for lung cancer immunotherapy. The detection of PD-L1$^+$ circulating tumor cells (CTCs) in patients with NSCLC treated with the PD-1 inhibitor nivolumab indicated that CTCs was a good liquid biopsy material associated with immunotherapy (65). At the American Association for Cancer Research Annual Meeting 2018, data from the CheckMate-227 trial indicated that the first-line treatment of nivolumab and ipilimumab in combination has improved progression-free survival (7.2 months) compared with chemotherapy (5.5 months) for patients with advanced NSCLC with high TMB (66). The importance of TMB detection makes blood-based TMB (bTMB) a considerable clinical biomarker (67). Using ctDNA, bTMB analysis may be performed more easily and at a higher rate, as indicated by the clinical outcomes stratified by bTMB in the POPLAR (clincaltrials.gov. no. NCT01903993) and OAK (clincaltrials.gov. no. NCT02008227) clinical trials (68), which assessed the efficacy of anti-PD-L1 CPI (atezolizumab) for recurrent advanced NSCLC. In this meeting, another trial (clinicaltrials.gov no. KEYNOTE-189) reported an improvement in overall survival by 8.8 months in the pembrolizumab-combination group and 4.9 months in the placebo-combination group across all PD-L1 categories that were evaluated, indicating the key role of PD-L1 detection in CPI therapy. In another trial (clincaltrials.gov. no. NCT02259621), TMB was used as an indicator of the pathological response to anti-PD-1 CPI therapy (38). At between 2 and 4 weeks after neoadjuvant nivolumab treatment, rapid expansion of mutation-associated neo-Ag-specific T-cell clones, from a primary tumor, along with a positive pathological assessment, was detected in peripheral blood in 8/9 patients assessed. A number of these clones were not detected prior to the administration of anti-PD-1 CPI (nivolumab).

4. TCR-engineered adoptive therapy targeting neo-Ags for lung cancer

Immunotherapies are developed to help strengthen the immune attack against tumor cells. One approach is CPIs, as aforementioned, and the other is TCR-engineered adoptive therapy (64,69). The increased sensitivity of the sequencing method allows for the detection of early-stage lung cancer by means of cfDNA analysis, as this technique will provide additional information about patients with cancer after a radiological screening method. Rizvi *et al* (70) indicated that a smoking signature and neo-Ags in the tumor were factors, which were associated with the response to anti-PD-1 CPI. It has been reported that tumor regression was associated with a neo-Ag-specific response by CD8$^+$ T cells (71). The accumulated evidence indicates that the genomic characteristics of a tumor may potentially assist in selecting and customizing immunotherapy. Consistent with these data, researchers have also been able to identify tumor-infiltrating CD8$^+$ T cells reactive to clonal neo-Ags in patients with NSCLC with homogenous and heterogeneous early-stage tumors (72). Adoptive T cell therapy was developed further in a number of ways on the basis of current knowledge. First, the CD8$^+$/PD-1$^+$

T cell subset, which was isolated and expanded from peripheral blood, was reinfused into the patient with cancer (41). Using high-throughput screening platforms, including NGS and high-performance liquid chromatography-mass spectrometry (HPLC-MS), neo-Ag-specific T cells in the PD-1$^+$ T cells may be identified, and may be used with their respective TCRs in immunotherapy (73). Another method of identification is the combination of HPLC-MS and sequencing, where novel neo-Ags can be identified (74). The focus of the study of Khodadoust *et al* (75) was on direct proteomic analysis of cancer MHC ligands and epitopes, using HPLC-MS rather than simply performing whole exome sequencing (WES) of DNA to identify tumor-associated non-synonymous somatic mutations. Neo-Ags are personalized antigens, except for certain common oncogene-specific antigens, including the KRAS proto-oncogene. A summary of TCR-engineered adoptive therapy targeting neo-Ags for lung cancer is presented in Fig. 3. The deep sequencing on tumor tissue, CTCs or ctDNA is used to determine the potential neo-Ags and TCR, in order to identify the sequences of the most dominant clonotypes within the PD-1$^+$ T cell subset. CTCs, a liquid biopsy material, can be enriched from the blood using a number of methods, including microfluidic isolation based on the epithelial cell adhesion molecule expression (76). This may be another way of obtaining neo-Ags from CTCs, based on NGS, since they provide more information about the primary or metastatic tumor sites.

5. Discussion and conclusions

Immunotherapy serves a key role in lung cancer therapy. CPIs have already been used for lung cancer therapy in various locations, including United States (77) and China (78). However, novel therapies targeting CPIs, including anti-CTLA4, anti-PD-1 and anti-PD-L1, are changing the prognosis of patients with advanced lung cancer. Randomized trials have reported improvements in OS compared with standard treatments, including chemotherapy and radiotherapy (6,11,13,77-81). Since there are biomarkers suitable for use in immunotherapy, a great deal of attention has been drawn to the assessment of PD-1 or PD-L1 expression in TME, challenged by the difficulty of accessing tissue samples, heterogeneity and the lack of gold-standard antibodies for IHC staining (82). WES for determination of TMB in liquid biopsy from patients with advanced NSCLC suggests that liquid biopsy-derived TMB may be used as a useful biomarker for predicting the CPI response, particularly in cases where tumor biopsy is not accessible or has been resampled (83). Theoretically, there are numerous potential biomarkers for immunotherapy in liquid biopsy; however, to the best of our knowledge, none has been identified to be reliable enough, particularly with respect to evaluating their efficiency or even their selection following drug resistance. It is important to identify liquid biopsy biomarkers for prognostic and response prediction associated with CPIs to guide future clinical decisions.

Successes with CPIs in the second-line treatment of NSCLCs have inevitably led to trials in the first-line setting (11). However, not all patients have reported an effective response. In clinical trials, patients who have presented with immunogenic tumors, including high TMB or neo-Ags, and pre-existing intratumoral immune infiltrate and immune escape ligands (i.e., PD-1/PD-L1) being targeted, seem to benefit the most from CPI therapy. As the first approved IHC assay for anti-PD-1 (pembrolizumab) in NSCLC, the PD-L1 (22C3) diagnostic (Dako PD-L1 IHC 22C3 pharmDx) (84,85) is still a key biomarker for the selection of patients with cancer (86). Pembrolizumab had been used as the first-line treatment (87), instead of cytotoxic chemotherapy, in patients with lung cancer whose proportion score for PD-L1 was ≥50% in TME (88). However, activating the immune system also presents with its own risks, since the immune CPIs give rise to grade 3/4 immune-associated adverse events (irAEs) with ipilimumab (15-25%), permbrolizumab (13%) and nivolumab (14%) (89,90). It is therefore necessary to elucidate the immune status in individual patients with cancer to identify a predictive method for these irAE risks. Biomarkers associated with these CPIs that predict efficacy, prognosis or risk of irAE risk may assist in the identification of patients who may benefit from these therapies. Biomarkers associated with clinical response prediction and the acquired resistance monitoring of lung cancer immunotherapy may be assessed in a dynamic manner using liquid biopsy based on blood samples, which would be beneficial to patients. However, extensive further investigation is required for the practical application of this treatment, largely due to the limitations of its sensitivity.

Although neo-Ag vaccines or TCR-engineered T cells targeting neo-Ags can be used for the majority of patients with cancer, the truly but rare tumor-specific T cells among the selected subset, may limit the therapeutic utility of T cell products (91). Therefore, more comprehensive technologies, including NGS, TCR sequencing and HPLC-MS are required. Furthermore, current methods for predicting tumor neo-Ags remain at an early stage and are limited by class I rather than class II MHC antigens (92). Additional efforts are required in the development of MHC class I- and class II-restricted neo-Ags as these will provide additional information about the immune surveillance in tumor development. The neo-Ag identification can be classified into direct and reverse identification using different techniques (93). The direct identification requires validation by exome and transcriptome sequencing data, whereas the MS-based reverse identification allows the identification of CD8$^+$ and CD4$^+$ T-cell neo-epitopes (29). However, to the best of our knowledge, the capacity of neo-Ag identification by direct identification has yet to be improved. It may eventually serve as a key tool in antigen discovery.

Lung cancer has entered the era of personalized immunotherapy (94,95). An improved understanding of the mechanisms of immunotherapies in patients with cancer will assist in the identification of biomarkers, suitable for the patients who will benefit the most from the treatment. Understanding the dynamics and diversity of these mechanisms will provide additional knowledge for when and how these therapeutic strategies should be utilized to prolong the effective response of immunotherapy in patients with NSCLC and therefore improving their outcomes.

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Authors' contributions

LLC and JW wrote the review, and have read and approved the final version of this manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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