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Posted Date: 2 October 2024

doi: 10.20944/preprints202410.0155.v1

Keywords: primary resistance; immunotherapy

 doi:10.20944/preprints202410.0155.v1

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Therapeutic Strategies to Target Immunotherapy Resistance in Non-Small Cell Lung Cancer (NSCLC)

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Abstract: Immunotherapy with programmed Cell Death Protein/Programmed Cell Death Ligand 1 (PD-1/PD-L1) targeting antibodies has transformed clinical outcomes for patients with non-small cell lung cancer (NSCLC) and has become an integral part of standard treatment regimens. However, the response to PD-1/PD-L1 inhibitors varies, and many tumors present with either primary or acquired resistance to immunotherapy. There are emerging data on potential mechanisms underlying resistance to immunotherapy which is derived mainly from either preclinical studies or secondary correlative analyses from clinical trials. Resistance to immunotherapy is complex and can present immediately after treatment initiation (primary resistance) or after initial clinical benefit (secondary or acquired resistance). The tumor microenvironment, particularly the presence, and activity of T cells, also significantly influences resistance with both tumor-extrinsic as well as tumor-intrinsic mechanisms such as lack of T-cell infiltration, insufficient neoantigens, or absence of an interferon signature identified as being associated with ICI resistance. Improved understanding of the molecular and immunologic mechanisms underlying immunotherapy resistance is essential for the future development of effective therapies targeting immunotherapy resistance. In this review, we discuss the emerging data identifying the molecular mechanisms of primary resistance to immunotherapy and explore potential therapeutic strategies to target these.

Keywords: primary resistance; immunotherapy

Introduction

Immunotherapy with programmed Cell Death Protein/Programmed Cell Death Ligand 1 (PD-1/PD-L1) targeting antibodies has transformed clinical outcomes for patients with non-small cell lung cancer (NSCLC) and has become an integral part of standard treatment regimens. The response to PD-1/PD-L1 inhibitors varies, and many tumors develop resistance to immunotherapy. Resistance to immunotherapy can present immediately after treatment initiation (primary resistance) or after initial clinical benefit (secondary or acquired resistance) [1]. The tumor microenvironment, particularly the presence, and activity of T cells, also significantly influences resistance to immunotherapy with both tumor-extrinsic as well as tumor-intrinsic mechanisms playing a role [2–5]. Improved understanding of the immunologic mechanisms underlying immunotherapy resistance is necessary for the future development of novel therapies targeting immunotherapy resistance. In this review, we discuss the potential therapeutic strategies to target immunotherapy resistance for the treatment of advanced NSCLC.

Immune Co-Stimulatory Antibodies

The T-cell mediated immune response against cancer cells is initiated through antigen recognition by the T cell receptor (TCR), which is controlled by a balance between co-inhibitory and stimulatory signals [6,7]. Although PD-1/PD-L1 and Cytotoxic T-lymphocyte associated protein 4 (CTLA4) pathways are the two most-studied immune checkpoints, many of the other immune regulatory checkpoints are currently being investigated. More recently, bispecific antibodies that

 doi:10.20944/preprints202410.0155.v1

simultaneously target PD-L1 and other immune regulatory molecules are also being investigated for patients with PD-1 resistant NSCLC [8]. One potential challenge for developing bispecific antibodies is that their efficacy may be limited if there is insufficient T-cell infiltration in immune-cold tumors [8]. Cadonilimab is a bispecific IgG-single-chain Fv fragment antibody that binds to PD-1 and CTLA-4 [9]. In a multicenter, phase Ib/II trial, patients with pre-treated NSCLC were enrolled in three cohorts: immunotherapy naïve; patients with primary resistance to immunotherapy; and patients with acquired resistance to immunotherapy [9]. Objective response rate (ORR) was 10% in PD-1 naïve cohort, but no responses were observed in the PD-1 resistant cohorts. Thus, cadonilimab demonstrated limited efficacy in patients with resistance to immunotherapy.

T cell immunoglobulin and ITIM domains (TIGIT) is a co-inhibitory receptor expressed on immune cells such as NK cells, effector T cells and regulatory T cells (Tregs) [10]. TIGIT is also co-expressed with PD-1 on exhausted T cells and may be a strategy to restore T-cell immunity [11]. Several anti-TIGIT antibodies are currently in development in PD-1 resistant NSCLC. A phase I trial with anti-TIGIT antibody, vibostolimab reported an ORR of 26% when combined with pembrolizumab in PD1-naive patients with NSCLC, but there was minimal efficacy in the PD-1 resistant cohort (ORR 3%) [12]. AZD2936 is a bispecific, humanized antibody targeting PD-1 and TIGIT. In an interim analysis of 80 patients enrolled in the ARTEMIDE-01 trial, AZD2936 showed an acceptable safety profile in patients with PD-1 resistant advanced NSCLC [13]. Among 76 evaluable patients, 3 patients had a partial response and 30 patients had stable disease. Further data is awaited (NCT04995523). HB0036 is a bispecific IgG1 antibody targeting both PD-L1 and TIGIT. In a phase I/II trial of HB0036 (NCT05417321) in patients with PD-1 resistant advanced solid tumors, no dose-limiting toxicity (DLTs) were observed. Disease control rate was 69% with durable response lasing more than 36 weeks in a patient with lung sarcomatoid carcinoma [14]. A phase I/II Study of HLX301, a recombinant humanized anti-PDL1 and anti-TIGIT bispecific antibody is currently enrolling patients with advanced solid tumors including NSCLC (NCT05390528).

Lymphocyte activation gene 3 (LAG3) is an inhibitory receptor expressed on activated T and NK cells which inhibits the function of CD8+ effector T cells as well as the function Tregs [15]. The phase II CITYSCAPE study evaluated anti-LAG3 antibody, tiragolumab plus atezolizumab as compared to placebo plus atezolizumab as first-line treatment in patients with PD-L1-positive advanced NSCLC. A higher efficacy was reported with the combination compared to atezolizumab alone (ORR 31% versus 16%, and median PFS 5.4 months versus 3.6 months; p=0.015) [16]. Based on this, the phase III SKYSCRAPER-01 study was initiated for patients with PD-L1-high advanced NSCLC but did not meet the co-primary PFS end point (NCT04294810). Eftilagimod alpha, a soluble LAG-3 protein, acts as an MHC class II agonist triggering activation of antigen-presenting cells (APC) and CD8 T-cells. In the TACTI-022 trial in 26 patients with PD-1 resistant disease, ORR and DCR were 8.3% and 33% respectively with eftilagimod alpha [17]. Most patients (~83%) showed either deceleration in tumor growth or shrinkage of target lesions. HLX26 is a novel humanized anti-LAG3 monoclonal antibody [18]. In a phase I trial, patients with refractory solid tumors including NSCLC were treated with escalating doses of HLX26 plus anti-PD1, serplulimab every 3 weeks [18]. In 9 treated patients (4 with NSCLC), three patients had best overall response (BOR) of stable disease. A phase I dose-finding study of RO7247669, an anti PD-1 and LAG-3 bispecific antibody is also currently enrolling (NCT04140500). TIM-3 is another inhibitory molecule like CTLA-4 and PD-1. AMBER trial (NCT02817633) is evaluating cobolimab as monotherapy and in combination with PD-1 inhibitors in advanced solid tumors [19]. COSTAR Lung (NCT04655976) is another trial in patients with PD-1 resistant NSCLC investigating cobolimab plus anti-PD1, dostarlimab and standard of care chemotherapy (docetaxel). AZD7789, an anti-PD-1 and anti-TIM-3 bispecific antibody is under investigation in a phase I/IIA trial in patients with advanced solid tumors (NCT04931654).

T-Cell Agonist Antibodies

Agonist antibodies against co-stimulatory molecules such as 4-1BB (CD137), OX40 (CD134), and ICOS (CD278) are being investigated in combination with anti-PD-1 immunotherapy but clinical responses have been modest to date [11]. The inducible co-stimulator (ICOS) of T cells is upregulated

 doi:10.20944/preprints202410.0155.v1

following initial T-cell priming and induces a signal through the PI3K/protein kinase B pathway, resulting in T-cell proliferation and survival [20]. The phase I/II ICONIC trial evaluated ICOS agonist, vopratelimab, alone and in combination with nivolumab in patients with advanced solid tumors. Vopratelimab resulted in a poor ORR of 1.4% and 2.3% in combination with nivolumab [21]. The prospective selection for ICOS positive tumors did not enrich the responses. A phase II trial was initiated in NSCLC (SELECT, NCT04549025) but eventually the drug program was discontinued. OX40 is an immune costimulatory receptor, expressed on activated CD4+ and CD8+ T cells, which promotes T cell proliferation and survival in the tumor microenvironment [22]. BGB-A445 is an agonist antibody that does not compete with endogenous OX40 ligand binding. In the dose-escalation part of the phase I trial (NCT04215978) of BGB-A445 alone or in combination with anti-PD1, tislelizumab patients with advanced solid tumors, ORR was 23% [22]. Further results from the trial are awaited. INBRX-106 is an agonistic, anti-OX40 antibody being investigated in combination with pembrolizumab in a phase I/II trial (NCT04198766). Multiple cohorts including PD-1 resistant NSCLC are enrolling. 4–1BB is a costimulatory receptor upregulated on tumor-infiltrating lymphocytes (TILs) which promote T-cell proliferation and activation [23]. INBRX-105 is a 4–1BB and PD-L1 bispecific antibody and cross-linking of PD-L1 to 4–1BB by INBRX-105 leads to conditional 4–1BB activation at sites of high PD-L1 expression, potentially limiting toxicities [23]. NCT03809624 is a phase I trial with multiple cohorts including in PD-1 resistant NSCLC but is no longer enrolling. GEN1046 is a PD-L1 and 4-1BB bispecific immunotherapy designed to act on both pathways by combining simultaneous and complementary PD-L1 blockade and conditional 4-1BB stimulation in one molecule [24]. A phase I trial of GEN1046 is ongoing with monotherapy as well as in combination with pembrolizumab in patients with PD-1 resistant NSCLC (NCT05117242).

Vaccine Therapy

Antigenic target vaccines are comprised of immunogenic tumor antigens or cells administered together with an immunoadjuvant to enhance anti-tumor immune response [25]. Tumor antigens may be tumor-associated antigens (TAA) or neoantigens arising from mutations in tumor DNA [25,26]. NEO-PV-01 is a personalized cancer vaccine consisting of up to 20 synthesized peptides of 14–35 amino acids derived from patient's mutated tumor DNA [27]. The NEO–PV–01 vaccine is administered after mixing with adjuvant poly-ICLC via injection subcutaneously. In a Phase I trial of NEO-PV-01 in combination with pemetrexed, carboplatin, and pembrolizumab as first-line therapy for advanced non-squamous NSCLC, 38 patients were treated with the regimen [27]. *De novo* neoantigen-specific CD4+ and CD8+ T cell responses were observed post-vaccination, and the regimen was tolerable. OSE2101 is a T-cell epitope-based cancer vaccine designed to induce cytotoxic T cells against five TAAs frequently overexpressed in NSCLC (HER-2/neu, CEA, MAGE 2, MAGE 3 and p53) [28]. TALANTE-1 was a two-step trial in patients with HLA-A2-positive PD-1 resistant advanced NSCLC (n=219) randomized to OSE2101 or chemotherapy (docetaxel or pemetrexed). In April 2020, a decision was taken to prematurely stop the accrual due to COVID-19. In the interim analysis, median OS favored OSE2101 over chemotherapy but was not statistically significant (P = 0.36) [28]. In the acquired resistance subgroup, OSE2101 significantly improved median OS versus chemotherapy (11.1 versus 7.5 months; P = 0.017). A phase III trial randomizing patients to OSE2101 vs chemotherapy is now planned in patients with HLA-A2 positive metastatic NSCC and acquired resistance to immunotherapy (ARTEMIA; NCT06472245).

CIMAvax-EGF is a vaccine comprising of a chemical conjugate between EGF and P64, a recombinant protein from *Neisseria meningitides* and an adjuvant [29]. Patients with PD1-naïve, pre-treated NSCLC were enrolled on a phase II trial and received CIMAvax-EGF every 2 weeks for 4 doses in combination with nivolumab followed by monthly maintenance with CIMAvax-EGF and nivolumab every 2 weeks [29]. The disease control rate was 47.6% with median OS of 11.9 months. CIMAvax-EGF is currently being investigated in combination with pembrolizumab as maintenance therapy after first line chemoimmunotherapy for NSCLC (NCT02955290). BNT116 is an intravenously administered RNA-lipoplex cancer vaccine comprising of six RNAs each encoding a TAA frequently expressed in NSCLC. LuCa-MERIT-1 is a Phase I trial of BNT116 alone or as

 doi:10.20944/preprints202410.0155.v1

combination therapy with cemiplimab, docetaxel, and/or carboplatin plus paclitaxel [30]. Preliminary results from the first 18 patients (n=13 monotherapy; n=5 cemiplimab added after Cycle 3) treated on the trial reported a tolerable safety profile. Adverse events (AEs) include pyrexia (67%), chills (50%), and vomiting (28%). Six of 10 evaluable patients had stable disease. Further enrolment on this trial is currently ongoing (NCT05142189). A Phase I trial is exploring the combination of cemiplimab with BNT116 as compared to cemiplimab alone for the first-line treatment of advanced NSCLC and PD-L1 ≥50% (NCT05557591). Another single institution, phase I study is investigating a pooled mutant-KRAS peptide vaccine with poly-ICLC adjuvant in combination with nivolumab and ipilimumab in the first line treatment of advanced NSCLC (NCT05254184).

Oncolytic Viruses (OV)

OVs are naturally occurring or genetically modified viruses that have been engineered to selectively cause tumor cell lysis while sparing normal host cells using several strategies [31]. Use of attenuated vectors or less-virulent strains of viruses may prevent acute or chronic infection [31]. OVs have demonstrated a tolerable safety profile in completed trials so far and have also demonstrated the ability to modify the tumor microenvironment and lyse tumor cells [31,32]. In NSCLC, clinical development of OVs has been limited by lack of significant benefit from OV monotherapy and by difficulty in achieving adequate viral load at tumor sites. One strategy to potentially overcome this is by exploring novel combinations with OVs, especially with checkpoint inhibitors and leveraging novel viral delivery systems [32]. Coxsackievirus A21 (Cavatak; CVA21), a naturally occurring human picornavirus, causes mild cold-like symptoms in humans. In the phase Ib STORM trial (NCT02043665; KEYNOTE-200), patients with advanced solid tumors were treated with CVA21 in escalating doses as either monotherapy or in combination with pembrolizumab [33]. There were no dose-limiting toxicities (DLTs), and all patients had detectable anti-CVA21 neutralizing antibodies by study day 22. ORR was 9% in the NSCLC expansion cohort (n=43) [34]. Although the virus was detected in tumor tissues and there was some increase in PDL1 expression on paired tumor biopsies, efficacy was not greater than that observed in previous studies with pembrolizumab monotherapy. CV301, a poxviral-based vaccine, has been evaluated in a phase I clinical trial and shown to be safe and immunologically active [35]. Patients with advanced non-squamous NSCLC received two priming doses of modified vaccinia Ankara-BN-CV301, followed by boosting doses of fowlpox-CV301 for up to 17 doses in combination with nivolumab or pembrolizumab [35]. Of 11 evaluable patients, 1 patient (9%) had a complete response, 1 patient (9%) had a partial response and 9 patients (82%) had stable disease. VSV-IFNβ-NIS is a vesicular stomatitis virus (VSV)-based OV being tested in combination with pembrolizumab in patients with refractory NSCLC or neuroendocrine cancers (NCT03647163).

Adenovirus is a non-enveloped, double-stranded DNA virus. It has a large linear genome which allows the incorporation of long DNA sequences, thus permitting multiple engineered modifications [31]. A phase II study investigated intratumoral injection of the oncolytic virus ADV/HSV-tk (adenovirus-mediated expression of herpes simplex virus thymidine kinase) followed by stereotactic body radiation therapy (SBRT) to the same tumor site in patients with both PD-1 naïve and PD-1 resistant stage IV NSCLC [36]. The ORR was 29% and the clinical benefit rate (CBR) was 62% in the PD-1 naïve group.

In the PD-1 resistant group, the ORR was 14% and the CBR was 64%. Gene-mediated cytotoxic immunotherapy (GMCI) is another approach in which an adenovirus-based vector expressing the thymidine kinase gene (aglatimagene besadenovec, AdV-tk) is locally administered followed by anti-viral drug valacyclovir. A phase I dose-escalation trial investigated GMCI in combination with intrapleural ADC-tk followed by chemotherapy in 19 patients with malignant pleural effusion from metastatic solid tumors (mesothelioma, NSCLC and breast cancer). There were no DLTs reported. Of the 4 patients with NSCLC, 3 patients had durable stabilization of disease with one patient continuing in follow-up for 29 months after therapy [37]. A phase II study of GMCI in combination with anti-PD1 immunotherapy for patients with PD-1 resistant NSCLC is currently enrolling (NCT04495153). MEM-288 is a conditionally replicative oncolytic adenovirus expressing human IFNβ and a

 doi:10.20944/preprints202410.0155.v1

recombinant membrane-stable form of CD40L [38]. In a phase I trial in patients with refractory solid tumors including NSCLC (n=11) [38], MEM-288 was administered intratumorally once every 3 weeks. Of the 10 evaluable patients, 4 had shrinkage of the injected tumor. Several patients also had stabilization or shrinkage of distal non-injected lesions. Biopsies showed decreased tumor cells, increase in CD8+ T cells, increase in T cell clonal diversity, and increase in TCF1+ stem-like CD8+ T cells. An expansion arm on this trial is currently enrolling patients with PD-L1 resistant NSCLC to test the combination of MEM-288 and anti-PD1 immunotherapy (NCT05076760).

Tumor Infiltrating Lymphocytes (TILs)

For TIL therapy, tumor-specific T cells are isolated via resection, activated in cytokines to restore their functionality, multiplied, and then infused back into the patient [39]. Before reintroducing the expanded TILs back into the patient, lymphodepleting chemotherapy is administered as well as IL2 to promote cell growth [39]. The main barriers to this approach are the need for a significant amount of fresh tissue, several weeks window before patient can start treatment, and the potential loss of tumor specificity *in vitro* [40]. In a phase I trial of TILs administered with nivolumab in 20 patients with PD-1 resistant, advanced NSCLC, three of the 13 evaluable patients had a confirmed response [41]. The trial reported complete responses in two patients and the responses were ongoing 1.5 years later. In a phase II multicenter study, lifileucel (LN-145), an autologous TIL therapy was investigated in patients with pre-treated metastatic NSCLC [42]. Lifileucel was manufactured using tumor tissue from different sites, but predominantly lung tissue was used. The ORR was 21.4% (6 of 28 patients). Responses occurred in tumors with profiles often resistant to immunotherapy, such as PD-L1-negative, low tumor mutational burden, and presence of STK11 mutation. Bone marrow suppression, hypotension, hypoxia, and fatigue were the most common AEs. These trials demonstrate that TILs can be a promising therapy for PD-1 resistant NSCLC. Several trials are currently investigating TIL therapy for refractory NSCLC (NCT02133196, NCT05681780, NCT05576077, NCT06060613).

Several novel approaches are also being utilized to improve the safety and efficacy of TILs. ATL001, an autologous clonal neoantigen reactive T cell (cNET) therapy is designed to target multiple clonal neoantigens expressed on tumor cells and absent from healthy tissue, on a personalized basis, in contrast to gene-modified approaches which are limited to single shared antigens that are not expressed on all cancer cells [43]. The product contains a mixed population of CD4+ and CD8+ T cells, both of which are important for maintenance of long-term cytotoxic responses [43]. CHIRON is a phase I/IIa study to evaluate the safety and clinical activity of ATL001 in patients with advanced NSCLC (NCT04032847).

Another strategy to improvise on TIL therapies is manipulation of the PD-1/PD-L1 axis to potentially enhance the efficacy as well as eliminate the immunotherapy adverse events. Common gene editing techniques being used include CRISPR-Cas9 and transcription activator-like effector nucleases (TALENs) [44]. A phase I/II trial of TALEN-mediated PD-1–inactivated TILs (IOV-4001) is currently ongoing with a plan to enroll 53 patients with metastatic melanoma and NSCLC (NCT05361174). LYL845 is an autologous TIL therapy produced with epigenetic reprogramming, which generates populations of tumor-reactive T cells with stem-like qualities and a more favorable phenotype (including CD8 skewing) [44]. A phase I trial is ongoing to investigate LYL845 in patients with advanced NSCLC, melanoma, and colorectal cancer (NCT05573035).

T-Cell Receptor (TCR) Therapy

TCR-based adoptive therapy utilizes genetically modified T cells that are directed against specific tumor markers [39,45]. TCR therapy usually utilizes TCRs restricted to common HLA alleles, such as HLA-A*02:01 [45]. Tumors targeted by TCR therapy need to express the targeted antigen as well as the corresponding antigen-restricting HLA allele. The first recombinant TCR therapy to be approved was afamitresgene autoleucel targeting MAGE-A4 for HLA-A*02 and MAGE-A4-expressing advanced synovial sarcoma in August 2024 [46]. A phase I trial investigated a novel affinity-enhanced NY-ESO-1-specific TCR in 9 patients with NY-ESO-1-expressing solid tumors R [47]. Of the 6 patients who received a dose of 5×10^9 cells, 3 patients demonstrated tumor responses.

 doi:10.20944/preprints202410.0155.v1

Three patients developed cytokine release syndrome (CRS) and 1 patient developed grade 3 lung injury. IMA203, an autologous TCR-engineered therapy uses a novel, pairing-enhanced TCR with high affinity and specificity for HLA-A*02:01-presented peptide related to PRAME, a potential target for multiple solid tumors including NSCLC. An ongoing trial is currently investigating this agent (ACTengine; NCT03686124). Preliminary results from 38 patients report that AEs were manageable with most common events being expected cytopenias (100%), CRS (92%, 3% grade 3) and ICANS (13%) [48]. Eleven of 18 (61%) patients showed an initial objective response at week 6 post infusion. Further enrolment is ongoing including cohorts combining TCR therapy with nivolumab. AFNT-211 is a TCR therapy consisting of autologous CD4+ and CD8+ T cells engineered to express HLA-A*11:01-restricted KRAS *G12V*-specific transgenic TCR, the wildtype CD8α/β coreceptor, and a FAS-41BB switch receptor [49]. A phase I/II trial with AFNT-211 is currently enrolling patients with KRAS *G12V* mutated solid tumors including NSCLC (NCT06105021) [49]. Similar TCR therapies are in progress in KRAS *G12V* mutated colon cancer and NSCLC (NCT06043713), TP53 *R175H* mutated solid tumors (NCT05877599) and KRAS *G12D* mutated tumors (NCT06218914).

Chimeric Antigen Receptor (CAR) T-Cell Therapy

CAR T-cell therapy, the adoptive transfer of engineered, CAR-expressing T lymphocyte was first approved in 2017 for the treatment of resistant lymphoma and acute lymphoblastic leukemia [50]. Since then, multiple CAR T therapies have been developed and are currently in investigation for the treatment of various malignancies. CAR T-cells are composed of three elements: an extracellular antigen-binding domain, an intracellular signaling domain which activates T cells, and a hinge that joins these two [51]. For NSCLC, CAR T-cell trials are leveraging targets such as mucin 1 (MUC 1), epidermal growth factor receptor (EGFR), ROR1 and mesothelin [51]. A phase I trial has been completed with anti-MUC1 CAR-T cells with PD-1 knockout through CRISPR-Cas9 in 20 patients with advanced NSCLC [52]. No grade 3-5 AEs and CRS were observed. Eleven of the 20 patients demonstrated stable disease as the best response to therapy. An ongoing trial is investigating MUC1 targeting CAR-T cells in solid tumors including NSCLC (NCT05239143). LYL797 is a ROR1-targeted CAR T-cell therapy, currently being investigated in patients with ROR1 expressing pre-treated NSCLC and triple-negative breast cancer (NCT05274451). A first-in-human, phase I study of autologous, mesothelin-targeted CAR T-cell therapy enrolled patients with metastatic lung cancer with pleural metastases as well as malignant pleural mesothelioma [53]. Intrapleural administration of CAR T cells was safe and well tolerated. Stable disease was sustained for ≥6 months in 8 patients; 2 exhibited complete metabolic response on PET scan.

Another potential target is epidermal growth factor receptor (EGFR), and a phase I clinical trial investigated EGFR targeting CAR-T cells generated by the piggyBac transposon system in advanced pre-treated NSCLC [54]. The piggyBac transposon system is a simpler, and alternative way to introduce CAR transgenes into T cells. Treatment was well tolerated with the most common AE being fever. After treatment, eight of nine patients with EGFR mutated NSCLC showed detectable EGFR-CAR T cells in their peripheral blood. One patient had a partial response to treatment lasting for more than 13 months, while six patients had stable disease. The median OS was 15.6 months. Another phase I trial is investigating EGFR targeting CAR-T cells modified by chemokine receptor type 5 (CXCR 5) in patients with advanced NSCLC (NCT05060796). The challenges to develop CAR-T therapy for NSCLC are poor infiltration of T cells in the tumor microenvironment, T cell exhaustion, and the heterogeneity of tumor antigens' expression [51]. Production of CAR-T cells is expensive and requires a significant amount of time which puts the patients with advanced NSCLC at risk for losing the window of opportunity to start treatment before the patients experience clinical deterioration [55]. One strategy to overcome some of these challenges is to use a split-CAR design that enables the engineering of multi-input CARs capable of Boolean-logic signal integration [56]. Some clinical trials for these logic-gated CAR T cells are ongoing such as EVEREST-1 which is evaluating A2B530 in tumors with enhanced CEA expression but loss of HLA-A*A02 expression (colon, pancreatic, and NSCLC tumors; NCT05736731). Another trial (EVEREST-2) is using a similar approach is investigating A2B694, an autologous logic-gated CAR T-cell therapy in tumors that express MSLN

 doi:10.20944/preprints202410.0155.v1

but have lost HLA-A*02 expression such as colorectal cancer, NSCLC, pancreatic cancer, ovarian cancer, mesothelioma, and other solid tumors that express MSLN and have lost HLA-A*02 expression (NCT06051695).

CAR NK Cell Therapy

NK cells kill target cells but lack a somatically rearranged and antigen-specific TCR as seen in CD8+ T cells [57]. The main sources of NK cells include peripheral blood, umbilical cord blood, NK cell lines (such as NK92), and induced pluripotent stem cells [57]. One barrier to CAR-NK cell development as a therapeutic strategy is the immune-suppressive effects of inhibitory receptors such as PD1 [58]. CAR-NK cells with a novel chimeric costimulatory converting receptor (CCCR), comprising mainly the extracellular domain of PD1, transmembrane and cytoplasmic domains of NKG2D, and the cytoplasmic domain of 41BB has been developed, that can switch the negative PD1 signal to an activating signal [58]. These CCCR-modified NK92 cells retain characteristics of NK cells and exhibited enhanced antitumor activity against human lung cancer cell lines. A trial has been conducted using this therapy in China in patients with NSCLC (NCT03656705). A phase I/II trial is currently enrolling patients with MUC1 positive solid tumors including NSCLC to investigate CAR-NK cell therapy (NCT02839954). Another trial is investigating the safety and efficacy of anti-Trop2 CAR-NK cell therapy combined with chemotherapy in refractory NSCLC (NCT06454890). NCT05334329 is a first-in-human, phase 1 dose-finding study of allogeneic, off-the shelf frozen and thawed Tumor-reactive and anti-PD-L1 co-stimulated killer cells (TRACK-NK) in patients with PD-1 resistant NSCLC [59]. TRACK-NK cells are PD-L1+ NK cells derived from cord blood and engineered to express soluble IL-15 (sIL-15) and ability to express high levels of tumor-reactive receptors that recognize "tumor stress" including DNAM-1, NKp30 and NKG2D. Other potential targets for CAR-NK cell therapy in NSCLC include B7-H3 and cMET and preclinical studies have shown potential with these therapies [60,61].

In conclusion, advances in the understanding of tumor biology as well as development of sophisticated engineering techniques have led to the development of several novel therapies targeting immunotherapy resistance in NSCLC such as personalized cellular therapy for lung cancer patients[44]. Further elucidation of the mechanisms underlying immunotherapy resistance is key to developing future treatment strategies. Several potential approaches to target these mechanisms of immunotherapy resistance have been tested but clinically significant benefit is yet to be seen. Many trials are still in early-phase investigation and several late-phase trials have not met the primary endpoint due to multiple factors such as heterogeneity in the mechanisms of immunotherapy resistance and lack of predictive biomarkers to select patients. Validation and integration of genomic and other biomarkers for future drug development is essential for effective targeting of immunotherapy resistance for the treatment of refractory NSCLC.

Table 1. Current trials in progress for PD1/PD-L1 resistant NSCLC.

Therapy name	Mechanism of action	NCT number
Co-stimulatory antibodies		
AZD2936	PD-L1×TIGIT bispecific antibody	NCT04995523 (ARTEMIDE)
HB0036	PD-L1×TIGIT bispecific antibody	NCT05417321
HLX301	PD-L1×TIGIT bispecific antibody	NCT05390528
RO7247669	PD-L1×LAG3 bispecific antibody	NCT04140500
Cobolimab	Anti-TIM3 antibody	NCT02817633 (AMBER)
Cobolimab	Anti-TIM3 antibody	NCT04655976 (COSTAR Lung)
AZD7789	PD-L1×TIM3 bispecific antibody	NCT04931654
T- cell agonists		
BGB-A445	OX40 agonist mAB	NCT04215978
INBRX-106	OX40 agonist mAB	NCT04198766

 doi:10.20944/preprints202410.0155.v1

ES102	OX40 agonist mAB	NCT04991506
GEN1046	PD-L1×4-1BB bispecific antibody	NCT05117242
Vaccine therapy		
OSE2101	Vaccine targeting 5 TAA overexpressed in NSCLC	NCT06472245 (ARTEMIA)
KRAS vaccine	Pooled mutant-KRAS peptide vaccine	NCT05254184
CIMAvax-EGF	Chemical conjugate between EGF and P64	NCT02955290
BNT116	RNA-lipoplex vaccine with 6 RNAs each encoding TAA expressed in NSCLC	NCT05142189 (LuCa-MERIT-1)
BNT116		NCT05557591
Oncolytic viruses		
Aglatimagene besadenovec (Adv-Tk)	Adenovirus-based vector expressing thymidine kinase gene	NCT04495153
MEM-288	Conditionally replicative adenovirus vector encoding transgenes for human IFNβ & recombinant chimeric form of CD40-ligand	NCT05076760
VSV-IFNβ-NIS	Oncolytic VSV expressing IFNβ and NIS	NCT03647163
TIL therapy		
LN-145	Autologous TILs	NCT04614103
	CD-40L-augmented TIL	NCT05681780
TBio-4101	Autologous TILs	NCT05576077 (STARLING)
OBX-115	IL-15 expressing TIL	NCT06060613
ATL001	clonal neoantigen reactive TILs	NCT04032847 (CHIROS)
IOV-4001	PD-1 inactivated TILs	NCT05361174
LYL845	Epigenetic reprogrammed TILs	NCT05573035
TCR therapy		
IMA203	PRAME specific TCR	NCT03686124 (ACTengine)
AFNT-211	HLA-A*11:01-restricted KRAS G12V TCR	NCT06105021
FH-A11KRASG12V-TCR	KRAS G12V specific TCR	NCT06043713
NT-112	HLA-C*08:02-restricted KRAS G12D TCR	NCT06218914
NT-175	HLA-A*02:01-restricted TP53 R175H TCR	NCT05877599
CAR-T		
P-MUC1C-ALLO1	Allogenic CAR-T targeting MUC1 expressing tumors	NCT05239143
LY797	ROR1 targeting CAR-T	NCT05274451
	CXCR5 modified EGFR targeted CAR-T	NCT05060796
A2B530	Logic-gated CAR-T (CEA expression, but loss of HLA-A*A02 expression)	NCT05736731 (EVEREST-1)
A2B694	Logic-gated CAR-T (MSLN expression, but loss of HLA-A*A02 expression)	NCT06051695 (EVEREST-2)
CAR-NK		
	anti-Trop2 CAR-NK	NCT06454890
	MUC1 targeting CAR-NK	NCT02839954
COH06	TRACK-NK	NCT05334329

mAB, monoclonal antibody; TAA, tumor associated antigen; VSV, vesicular stomatitis virus; IFNβ, interferon-beta; NIS, sodium iodide symporter; TRACK-NK, tumor-reactive and anti-PD-L1 co-stimulated killer cells.

 doi:10.20944/preprints202410.0155.v1

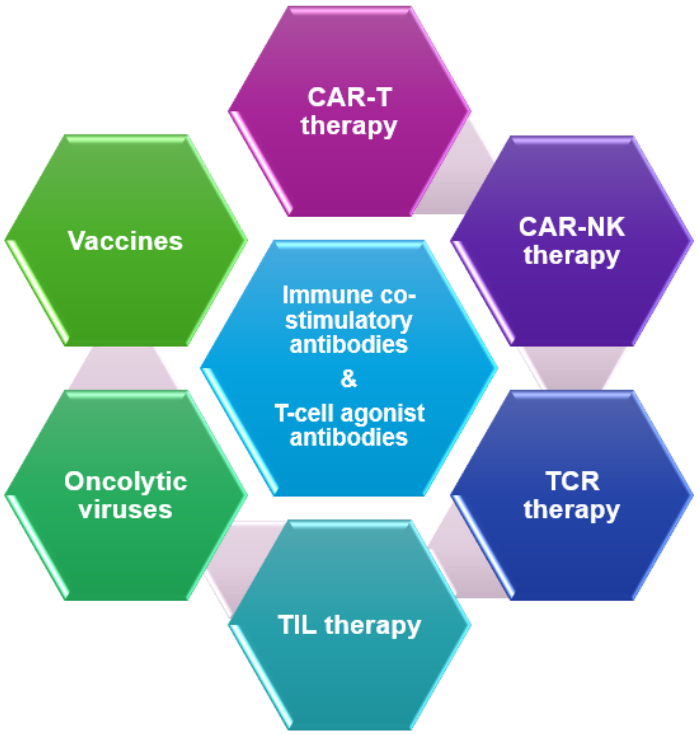

Figure 1. Therapeutic approaches for PD1/PD-L1 resistant NSCLC.

References

1. Kluger, H.M., et al., Defining tumor resistance to PD-1 pathway blockade: recommendations from the first meeting of the SITC Immunotherapy Resistance Taskforce. J Immunother Cancer, 2020. **8**(1).
2. Uryvaev, A., et al., The role of tumor-infiltrating lymphocytes (TILs) as a predictive biomarker of response to anti-PD1 therapy in patients with metastatic non-small cell lung cancer or metastatic melanoma. Med Oncol, 2018. **35**(3): p. 25.
3. Tumeh, P.C., et al., PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature, 2014. **515**(7528): p. 568-71.
4. Snyder, A., et al., Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med, 2014. **371**(23): p. 2189-2199.
5. Ayers, M., et al., IFN-gamma-related mRNA profile predicts clinical response to PD-1 blockade. J Clin Invest, 2017. **127**(8): p. 2930-2940.
6. Wang, M., et al., Immune checkpoint blockade and its combination therapy with small-molecule inhibitors for cancer treatment. Biochim Biophys Acta Rev Cancer, 2019. **1871**(2): p. 199-224.
7. Bluthgen, M.V., N. Baste, and G. Recondo, *Immunotherapy combinations for the treatment of patients with solid tumors.* Future Oncol, 2020. **16**(23): p. 1715-1736.
8. Li, T., et al., The enhanced antitumor activity of bispecific antibody targeting PD-1/PD-L1 signaling. Cell Commun Signal, 2024. **22**(1): p. 179.
9. Zhao, Y., et al., A multicenter, open-label phase Ib/II study of cadonilimab (anti PD-1 and CTLA-4 bispecific antibody) monotherapy in previously treated advanced non-small-cell lung cancer (AK104-202 study). Lung Cancer, 2023. **184**: p. 107355.
10. Yu, X., et al., The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. Nat Immunol, 2009. **10**(1): p. 48-57.
11. Martin, C. and D. Enrico, Current and novel therapeutic strategies for optimizing immunotherapy outcomes in advanced non-small cell lung cancer. Front Oncol, 2022. **12**: p. 962947.
12. Niu, J., et al., First-in-human phase 1 study of the anti-TIGIT antibody vibostolimab as monotherapy or with pembrolizumab for advanced solid tumors, including non-small-cell lung cancer(☆). Ann Oncol, 2022. **33**(2): p. 169-180.
13. Rohrberg, K.S., et al., Safety, pharmacokinetics (PK), pharmacodynamics (PD) and preliminary efficacy of AZD2936, a bispecific antibody targeting PD-1 and TIGIT, in checkpoint inhibitor (CPI)-experienced advanced/metastatic non-small-cell lung cancer (NSCLC): First report of ARTEMIDE-01. Journal of Clinical Oncology, 2023. **41**(16_suppl): p. 9050-9050.

 doi:10.20944/preprints202410.0155.v1

14. Sun, Y., et al., A phase I/II, open-label, multicenter study to evaluate the safety, pharmacokinetics, pharmacodynamics, and efficacy of HB0036 in patients with advanced solid tumors. Journal of Clinical Oncology, 2024. **42**(16_suppl): p. e14504-e14504.
15. Triebel, F., et al., LAG-3, a novel lymphocyte activation gene closely related to CD4. J Exp Med, 1990. **171**(5): p. 1393-405.
16. Cho, B.C., et al., Tiragolumab plus atezolizumab versus placebo plus atezolizumab as a first-line treatment for PD-L1-selected non-small-cell lung cancer (CITYSCAPE): primary and follow-up analyses of a randomised, double-blind, phase 2 study. Lancet Oncol, 2022. **23**(6): p. 781-792.
17. Majem, M., et al., 11MO Final data from a phase II study (TACTI-002) of eftilagimod alpha (soluble LAG-3) and pembrolizumab in 2nd-line metastatic NSCLC pts resistant to PD-1/PD-L1 inhibitors. Journal of Thoracic Oncology, 2023. **18**(4, Supplement): p. S43-S44.
18. Wu, Y., et al., 80P Safety, preliminary efficacy, and pharmacokinetics of HLX26 plus serplulimab in advanced solid tumours: An open-label, dose-escalation phase I study. Annals of Oncology, 2023. **34**: p. S1497.
19. Falchook, G.S., et al., Phase 1 trial of TIM-3 inhibitor cobolimab monotherapy and in combination with PD-1 inhibitors nivolumab or dostarlimab (AMBER). Journal of Clinical Oncology, 2022. **40**(16_suppl): p. 2504-2504.
20. Hutloff, A., et al., ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. Nature, 1999. **397**(6716): p. 263-6.
21. Yap, T.A., et al., First-in-Human Phase I/II ICONIC Trial of the ICOS Agonist Vopratelimab Alone and with Nivolumab: ICOS-High CD4 T-Cell Populations and Predictors of Response. Clin Cancer Res, 2022. **28**(17): p. 3695-3708.
22. Desai, J., et al., A phase 1 study of the OX40 agonist, BGB-A445, with or without tislelizumab, an anti-PD-1 monoclonal antibody, in patients with advanced solid tumors. Journal of Clinical Oncology, 2023. **41**(16_suppl): p. 2574-2574.
23. Park, J.C., et al., 742 Phase 1/2 study of the bispecific 4–1BB and PD-L1 antibody INBRX-105 alone and in combination with pembrolizumab in select solid tumors. Journal for ImmunoTherapy of Cancer, 2023. **11**(Suppl 1): p. A836-A837.
24. Muik, A., et al., Preclinical Characterization and Phase I Trial Results of a Bispecific Antibody Targeting PD-L1 and 4-1BB (GEN1046) in Patients with Advanced Refractory Solid Tumors. Cancer Discov, 2022. **12**(5): p. 1248-1265.
25. Tagliamonte, M., et al., *Antigen-specific vaccines for cancer treatment.* Hum Vaccin Immunother, 2014. **10**(11): p. 3332-46.
26. Malhotra, J. and J.M. Mehnert, Use of tumor cell lysate to develop peptide vaccine targeting cancer-testis antigens. Transl Lung Cancer Res, 2021. **10**(11): p. 4049-4052.
27. Awad, M.M., et al., Personalized neoantigen vaccine NEO-PV-01 with chemotherapy and anti-PD-1 as first-line treatment for non-squamous non-small cell lung cancer. Cancer Cell, 2022. **40**(9): p. 1010-1026 e11.
28. Besse, B., et al., Randomized open-label controlled study of cancer vaccine OSE2101 versus chemotherapy in HLA-A2-positive patients with advanced non-small-cell lung cancer with resistance to immunotherapy: ATALANTE-1. Ann Oncol, 2023. **34**(10): p. 920-933.
29. Frascati, R., et al., Final results from a phase II trial of CIMAvax-EGF and nivolumab as second-line (2L) therapy after platinum-based chemotherapy in advanced non-small cell lung cancer (NSCLC). Journal of Clinical Oncology, 2023. **41**(16_suppl): p. 9135-9135.
30. Deme, D., et al., 597 Preliminary results from LuCa-MERIT-1, a first-in-human Phase I trial evaluating the fixed antigen RNA vaccine BNT116 in patients with advanced non-small cell lung cancer. Journal for ImmunoTherapy of Cancer, 2023. **11**(Suppl 1): p. A679-A679.
31. Kaufman, H.L., F.J. Kohlhapp, and A. Zloza, *Oncolytic viruses: a new class of immunotherapy drugs.* Nat Rev Drug Discov, 2015. **14**(9): p. 642-62.
32. Malhotra, J. and E.S. Kim, *Oncolytic Viruses and Cancer Immunotherapy.* Curr Oncol Rep, 2023. **25**(1): p. 19-28.
33. Pandha, H.S., et al., Keynote-200 phase 1b: A novel combination study of intravenously delivered coxsackievirus A21 and pembrolizumab in advanced cancer patients. Journal of Clinical Oncology, 2017. **35**(15_suppl): p. TPS3108-TPS3108.
34. Rudin, C.M., et al., Phase 1, open-label, dose-escalation study on the safety, pharmacokinetics, and preliminary efficacy of intravenous Coxsackievirus A21 (V937), with or without pembrolizumab, in patients with advanced solid tumors. J Immunother Cancer, 2023. **11**(1).
35. Rajan, A., et al., Phase 1 trial of CV301 in combination with anti-PD-1 therapy in nonsquamous non-small cell lung cancer. Int J Cancer, 2023. **152**(3): p. 447-457.
36. Guerrero, C., et al., Stereotactic body radiation therapy and in situ oncolytic virus therapy followed by immunotherapy in metastatic non-small cell lung cancer. Journal of Clinical Oncology, 2021. **39**(15_suppl): p. 9115-9115.

 doi:10.20944/preprints202410.0155.v1

37. Aggarwal, C., et al., Phase I Study of Intrapleural Gene-Mediated Cytotoxic Immunotherapy in Patients with Malignant Pleural Effusion. Mol Ther, 2018. **26**(5): p. 1198-1205.
38. Saltos, A.N., et al., A phase 1 first-in-human study of interferon beta (IFNβ) and membrane-stable CD40L expressing oncolytic virus (MEM-288) in solid tumors including non–small-cell lung cancer (NSCLC). Journal of Clinical Oncology, 2023. **41**(16_suppl): p. 2569-2569.
39. Mountzios, G., et al., Beyond Chemoimmunotherapy in Advanced Non-Small Cell Lung Cancer: New Frontiers, New Challenges. Am Soc Clin Oncol Educ Book, 2024. **44**(3): p. e432526.
40. Poschke, I.C., et al., The Outcome of Ex Vivo TIL Expansion Is Highly Influenced by Spatial Heterogeneity of the Tumor T-Cell Repertoire and Differences in Intrinsic In Vitro Growth Capacity between T-Cell Clones. Clin Cancer Res, 2020. **26**(16): p. 4289-4301.
41. Creelan, B.C., et al., Tumor-infiltrating lymphocyte treatment for anti-PD-1-resistant metastatic lung cancer: a phase 1 trial. Nat Med, 2021. **27**(8): p. 1410-1418.
42. Schoenfeld, A.J., et al., Lifileucel, an Autologous Tumor-Infiltrating Lymphocyte Monotherapy, in Patients with Advanced Non-Small Cell Lung Cancer Resistant to Immune Checkpoint Inhibitors. Cancer Discov, 2024. **14**(8): p. 1389-1402.
43. Robertson, J., M. Salm, and M. Dangl, Adoptive cell therapy with tumour-infiltrating lymphocytes: the emerging importance of clonal neoantigen targets for next-generation products in non-small cell lung cancer. Immunooncol Technol, 2019. **3**: p. 1-7.
44. Katiyar, V., J. Chesney, and G. Kloecker, Cellular Therapy for Lung Cancer: Focusing on Chimeric Antigen Receptor T (CAR T) Cells and Tumor-Infiltrating Lymphocyte (TIL) Therapy. Cancers (Basel), 2023. **15**(14).
45. Tsimberidou, A.M., et al., T-cell receptor-based therapy: an innovative therapeutic approach for solid tumors. J Hematol Oncol, 2021. **14**(1): p. 102.
46. D'Angelo, S.P., et al., Afamitresgene autoleucel for advanced synovial sarcoma and myxoid round cell liposarcoma (SPEARHEAD-1): an international, open-label, phase 2 trial. Lancet, 2024. **403**(10435): p. 1460-1471.
47. Ishihara, M., et al., NY-ESO-1-specific redirected T cells with endogenous TCR knockdown mediate tumor response and cytokine release syndrome. J Immunother Cancer, 2022. **10**(6).
48. Wermke, M., et al., Abstract PR018: IMA203 TCR-T targeting PRAME demonstrates potent anti-tumor activity in patients with different types of metastatic solid tumors. Molecular Cancer Therapeutics, 2023. **22**(12_Supplement): p. PR018-PR018.
49. Mitchell, S., et al., AFNT-211: A phase 1 study of autologous CD4+ and CD8+ T cells engineered to express a high avidity HLA-A*11:01-restricted, KRAS G12V-specific, transgenic TCR, a CD8α/β coreceptor, and a FAS41BB switch receptor in patients with advanced/metastatic solid tumors. Journal of Clinical Oncology, 2024. **42**(16_suppl): p. TPS8650-TPS8650.
50. Chen, Y.J., B. Abila, and Y. Mostafa Kamel, *CAR-T: What Is Next?* Cancers (Basel), 2023. **15**(3).
51. Ma, H.Y., et al., *Advances in CAR T Cell Therapy for Non-Small Cell Lung Cancer.* Curr Issues Mol Biol, 2023. **45**(11): p. 9019-9038.
52. Lin, Y., et al., 35O - Phase I clinical trial of PD-1 knockout anti-MUC1 CAR-T cells in the treatment of patients with non-small cell lung cancer. Annals of Oncology, 2019. **30**: p. xi12.
53. Adusumilli, P.S., et al., A Phase I Trial of Regional Mesothelin-Targeted CAR T-cell Therapy in Patients with Malignant Pleural Disease, in Combination with the Anti-PD-1 Agent Pembrolizumab. Cancer Discov, 2021. **11**(11): p. 2748-2763.
54. Zhang, Y., et al., Phase I clinical trial of EGFR-specific CAR-T cells generated by the piggyBac transposon system in advanced relapsed/refractory non-small cell lung cancer patients. J Cancer Res Clin Oncol, 2021. **147**(12): p. 3725-3734.
55. Chocarro, L., et al., *CAR-T Cells for the Treatment of Lung Cancer.* Life (Basel), 2022. **12**(4).
56. Chen, Y.Y., *Increasing T Cell Versatility with SUPRA CARs.* Cell, 2018. **173**(6): p. 1316-1317.
57. Liu, F., et al., Advances in CAR-NK cell therapy for lung cancer: is it a better choice in the future? Front Oncol, 2024. **14**: p. 1390006.
58. Lu, C., et al., A novel chimeric PD1-NKG2D-41BB receptor enhances antitumor activity of NK92 cells against human lung cancer H1299 cells by triggering pyroptosis. Mol Immunol, 2020. **122**: p. 200-206.
59. Villalona-Calero, M.A., et al., A phase 1 trial of umbilical cord blood–derived tumor-reactive PD-L1+ natural killer cells engineered to express soluble IL-15 (TRACK-NK) in patients with non–small-cell lung cancer (NSCLC) refractory to PD-1/PD-L1 inhibitors. Journal of Clinical Oncology, 2023. **41**(16_suppl): p. TPS2665-TPS2665.
60. Yang, S., et al., Targeting B7-H3 Immune Checkpoint With Chimeric Antigen Receptor-Engineered Natural Killer Cells Exhibits Potent Cytotoxicity Against Non-Small Cell Lung Cancer. Front Pharmacol, 2020. **11**: p. 1089.
61. Peng, Y., et al., Engineering c-Met-CAR NK-92 cells as a promising therapeutic candidate for lung adenocarcinoma. Pharmacol Res, 2023. **188**: p. 106656.

 doi:10.20944/preprints202410.0155.v1