Clinical Perspectives to Overcome Acquired Resistance to Anti–Programmed Death-1 and Anti–Programmed Death Ligand-1 Therapy in Non-Small Cell Lung Cancer

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INTRODUCTION

Immune checkpoint inhibitors (ICIs) that target cytotoxic T lymphocyte associated antigen-4 (CTLA-4), PD-1, and PD-L1 receptors have been shown to have beneficial therapeutic effects in lung cancer (Steven et al., 2016). ICIs are the first-line treatment for non-small cell lung cancer (NSCLC) with positive PD-L1 expression (Ettinger et al., 2019). However, only 20% to 30% of NSCLC patients are sensitive to anti–PD-1/PD-L1 therapy, and most patients experience resistance to immunotherapy (Pourmir et al., 2020). Acquired resistance is defined as disease progression within 6 months after a period of clinical benefit (Remon et al., 2020; Sharma et al., 2017). The mechanisms of acquired resistance remain to be fully elucidated, as research on treatment strategies to overcome resistance to approved immunotherapies is ongoing (Bagchi et al., 2021). Here, we discuss the mechanisms of acquired resistance to anti–PD-1/PD-L1 therapy in NSCLC, including loss of immunogenic neoantigens, upregulation of alternate immune checkpoint receptors, increase in immunosuppressive cells, cytokines, and immunoregulatory molecules in the tumor microenvironment, and epigenetic modifications. In addition, we address the potential therapeutic targets and ongoing clinical trials, focusing mainly on NSCLC.
addition, we have summarized the potential therapeutic targets and ongoing clinical trials.

**MECHANISMS OF ACQUIRED RESISTANCE TO ANTI–PD-1/PD-L1**

### Loss of immunogenic neoantigen

**B2M and MHC defects**

Defects in beta-2-microglobulin (B2M) or major histocompatibility complex (MHC) molecules can cause decreased neoantigen presentation (Mariathasan et al., 2018; Sucker et al., 2014). B2M stabilizes the alpha subunits of the MHC-I protein, and a mutation in the B2M gene results in loss of neoantigen surface expression (Gettinger et al., 2017; Zaretzky et al., 2016). In NSCLC, acquired homozygous loss of B2M results in a lack of MHC-I expression on the cell surface, which results in acquired resistance to PD-1 therapy (Gettinger et al., 2017). In addition to loss of heterozygosity, deletions or point mutations in the B2M gene have been found to be important pathways for both primary and acquired resistance to ICIs (Gettinger et al., 2017; Pereira et al., 2017).

**Defects in the IFN-γ pathway**

Activated T cells and natural killer (NK) T cells release interferon-gamma (IFN-γ) into the tumor microenvironment and affect immune reactions through the downstream enzymes Janus kinase 1 and 2 (JAK1 and JAK2) and signal transducer and activators of transcription (STATs) (Taube et al., 2012). IFN-γ stimulates antigen production, upregulation of PD-L1 expression in tumor cells, and production of T cell-attracting chemokines (Abiko et al., 2015). Deficiencies in IFN-γ, JAK1/2, or STATs prevent IFN-γ signaling and consequently result in downregulation of T cell infiltration, and decrease in PD-L1 and MHC-I expression (Bach et al., 1997; Sucker et al., 2017). In patients with melanoma, JAK1- or JAK2-inactivating mutations lead to acquired resistance to anti-PD-1 therapy via inhibition of the IFN-γ pathway and PD-L1 expression (Shin et al., 2017). Loss of PD-L1 expression is associated with less effective PD-1 blocking (Ren et al., 2020). Other IFN-γ pathway-related gene mutations, such as deletion of IFN-γ receptor 1 and 2 (IFNγR1 and 2), STAT2, JAK1, and JAK2, also result in acquired resistance in melanoma (Manguso et al., 2017; Ren et al., 2020).

Targeting downstream factors, such as JAK1/2 and STAT, is a possible treatment option to overcome acquired resistance to anti-PD-1 therapy in lung cancer (Table 1). A combination of JAK-STAT or vascular endothelial growth factor (VEGF) inhibitors and immune checkpoint therapy can help control tumor growth in phosphatase and tensin homolog (PTEN)-mediated acquired resistance to immune checkpoint monotherapy (Peng et al., 2016; Toso et al., 2014). Dual inhibition of the JAK1,2/PD-L1 and STAT3/PD-L1 signaling pathways led to better immune cytolytic activity of NK cells toward hypoxia-induced castrate-resistant prostate cancer (CRPC) cells (Xu et al., 2018). However, the combination of anti-PD-1 therapy with JAK/STAT inhibitors has also been shown to reduce anti-tumor effects and tumor infiltrating lymphocyte (TIL) numbers (Ashizawa et al., 2019).

### Upregulation of other immune checkpoint receptors

Immune checkpoint receptors are upregulated as a compensatory mechanism after immunotherapy. These mechanisms include T cell exhausation, proliferation, migration, and cytokine secretion by CD8+ T cells (Thommen et al., 2015; Topalian et al., 2015). Immune checkpoints such as lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and mucin domain 3 (TIM-3), and T cell immunoreceptors with Igl and

#### Table 1. Mechanisms of acquired resistance and potential therapeutic approaches

| Resistance mechanisms | Description of resistance mechanisms | Potential therapeutic approaches |
|-----------------------|--------------------------------------|--------------------------------|
| Loss of immunogenic neoantigen | Defects in IFN-γ pathway | STING agonist, JAK inhibitor, STAT inhibitor |
| Upregulation of alternate immune checkpoint receptors | Compensatory upregulation of inhibitory receptors (LAG-3, TIM-3, TIM3, TIGIT, BTLA, VISTA, SIGLEC9) | Blockade of alternate co-inhibitory immune checkpoint receptors: LAG-3, TIM-3, TIGIT, BTLA, VISTA, SIGLEC9 |
| Immunosuppressive cells and immunoregulative molecules in tumor microenvironment | Increased immunosuppressive cells (Treg, MDSC, M2 macrophage) | Immune stimulatory agents: OX40, ICOS |
| | Elevated immunosuppressive cytokines (TGF-β, VEGF, IL-6/8) | CSF1R inhibitor, TGF-β inhibitor |
| | Immune regulating molecules: adenosine pathway, IDO1, B7-H4 | TGF-β inhibitor, VEGF inhibitor, IL-1β inhibitor, IL-6/8 inhibitor |
| Epigenetic modification | Tumor suppressor, apoptosis gene modification | A2AR inhibitor/anti-CD73, IDO inhibitor, B7-H4 inhibitor |
| | Stability of chromatin remodeling complexes | Epigenetic modifiers: DNMTi, HMTi, HDACi |

**IFN-γ**, interferon-γ; **STING**, stimulator of IFN genes; **JAK**, Janus kinase; **STAT**, signal transducer and activators of transcription; **LAG-3**, lymphocyte-associated gene 3; **TIM-3**, T-cell immunoglobulin and mucin domain-3; **TIGIT**, T-cell immunoglobulin and ITIM domain; **BTLA**, B and T-lymocyte attenuator; **VISTA**, V-domain immunoglobulin suppressor of T-cell activation; **SIGLEC9**, sialic acid binding Ig-like lectin 9; **ICOS**, inducible T-cell costimulator; **Treg**, regulatory T-cell; **MDSC**, myeloid-derived suppressor cell; **CSF1R**, colony stimulating factor 1 receptor; **TGF-β**, transforming growth factor-β; **VEGF**, vascular endothelial growth factor; **IL**, interleukin; **IDO**, indoleamine 2,3-dioxygenase; **A2AR**, adenosine A2A receptor; **DNMTi**, DNA methyltransferase inhibitor; **HMTi**, histone methyltransferase inhibitor; **HDACi**, histone deacetylase inhibitor.

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ITIM domains (TIGIT) create an immunosuppressive environment (Fig. 1, Table 1) (Toor et al., 2020). LAG-3 is expressed on TILs, and dual blockade of LAG-3 and PD-1 resulted in synergistic anti-tumor effects in preliminary models (Hellmann et al., 2016). TIM-3 was upregulated in both CD4+ and CD8+ T cells in patients with lung cancer refractory to anti–PD-1 therapy (Koyama et al., 2016). Similarly, TIGIT expression on tumor antigen-specific CD8+ T cells was observed in patients with melanoma after anti–PD-1 treatment (Chauvin et al., 2015).

Other immune checkpoint receptors such as B and T lymphocyte attenuator (BTLA), V-domain immunoglobulin-containing suppressor of T cell activation (VISTA), and sialic acid-binding Ig-like lectin 9 (SIGLEC9) are also potential treatment targets (Galon and Bruni, 2019). Similarly, immune stimulatory agents such as OX40 and inducible T cell costimulatory (ICOS) agonists enhance T cell expansion and effector functions by controlling the tumor suppressive function of regulatory T cells (Tregs) (Hu-Lieskovan and Ribas, 2017; Mahoney et al., 2015).

Suppressive tumor microenvironment

Immunosuppressive cells

In patients refractory to anti–PD-1 therapy, decreased T cell effector function is associated with an increase in immunosuppressive cells such as Tregs, myeloid-derived suppressor cells (MDSCs), and tumor associated macrophages (TAM) (Fig. 1, Table 1) (Arlauckas et al., 2017). Tregs directly inhibit effector T cells (Teff) or produce inhibitory cytokines, such as interleukin (IL)-10, IL-35, and transforming growth factor-β (TGF-β), which suppress CD8+ T cells, resulting in acquired resistance to ICIs (Sakaguchi et al., 2008; Saleh and Elkord, 2019). MDSCs induce acquired resistance to ICIs via direct action on T cells, promotion of tumor angiogenesis, and recruitment of immune suppressive cells to the tumor microenvironment (Hou et al., 2020). MDSCs in the tumor microenvironment are related to a lack of response to immunotherapy (Meyer et al., 2014). M2 macrophages reshape the tumor microenvironment into a pro-tumorigenic environment (Chanmee et al., 2014). The colony-stimulating growth factor 1 receptor (CSF1R) plays a critical role in differentiation, pro-

Fig. 1. Immune suppressive and immune stimulatory cell-favored niche. The immune suppressive environment (left) shows the 1) immune suppressive cells including Tregs and MDSCs, 2) the expression of immune suppressive cytokines, and 3) upregulation of immune checkpoint receptors such as TIGIT, LAG-3, and TIM-3 by T cells. The immune stimulatory environment includes PD-1 expression by T cells (right). The immune suppressive cell-favored niche does not respond well to ICIs, while the immune stimulatory responds favorably to ICIs.

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liferation, and survival of the mononuclear phagocyte system and macrophages (Stanley and Chitu, 2014). Blocking CSF1R results in a decrease in tumor-associated macrophages, and addition of CSF1R inhibitor with PD1 and CTLA4 antagonists improves the response to ICIs in pancreatic cancer mouse models (Zhu et al., 2014), suggesting CSF1R inhibitor as a therapeutic approach for immunotherapy resistance.

**Immunosuppressive cytokines**

The upregulation of the TGF-β pathway promotes the immunosuppressive effects of Tregs (Table 1) (Neel et al., 2012). Inhibition of TGF-β provided a better anti-tumor response to ICIs in a colorectal cancer model, followed by application of a TGF-β inhibitor with or without anti–CTLA-4 or radiation therapy (Fig. 1) (Hanks et al., 2014; Vanpouille-Box et al., 2015).

VEGF signaling activates the infiltration of Tregs into the tumor microenvironment and induces exhaustion of cytotoxic T lymphocytes (CTLs) by increasing inhibitory receptor expression (Voron et al., 2015). Patients with anti-PD-1 resistance have higher levels of VEGF than anti-PD-1 responsive patients (Chen et al., 2016). Combined anti-VEGF/anti-PD-L1 therapy has been shown to have beneficial outcomes in small cell lung cancer (SCLC) mouse models (Meder et al., 2018). These results suggest that the addition of VEGF inhibitors may improve the response to immunotherapy.

IL-6 and IL-8 are proinflammatory cytokines that are found in the tumor microenvironment. IL-6 decreases PD-L1 and MHC class 1 expression, leading to tumor evasion and ICI therapy resistance (García-Díaz et al., 2017). IL-8 modulates chemotaxis of neutrophils, resulting in pro-tumorigenic effects (Alfaro et al., 2016). High concentrations of IL-8 inhibit T cell function and antigen presentation, thereby promoting resistance to ICI therapy (Yuen et al., 2020).

**Immunoregulative molecules**

Immunoregulatory molecules such as adenosine, indoleamine 2,3-dioxygenase 1 (IDO1), and B7-H4 contribute to immunosuppression, which is associated with ICI resistance (Table 1) (Platten et al., 2015; Zang et al., 2003; Zhang et al., 2004). Adenosine inhibits effector T cells and increases Tregs via adenosine A2A receptor (A2AR) binding, leading to a decrease in NK cell maturation and its action (Young et al., 2018). Blocking CD73 or A2AR prevents adenosine signaling and improves the response of tumor cells to anti–PD-1 therapy (Vijayan et al., 2017). IDO1 is an enzyme that converts tryptophan to kynurenine. Consumption of tryptophan and accumulation of kynurenine activates Teff and Tregs, and promotes Treg cell formation (Ricciuti et al., 2019). Combination of IDO inhibitors with ICI therapy enhances the TIL function and number in the tumor microenvironment (Spranger et al., 2014). B7-H4 binds to T cells and inhibits their proliferation, cytotoxic action, and interleukin secretion by T cells (Zang et al., 2003). In patients with advanced NSCLC, high expression of B7-H4 is associated with tumor progression and tumor-related death risks (Genova et al., 2019). The effect of B7-H4 on immunotherapy resistance remains to be fully elucidated.

**Epigenetic modification**

Epigenetic modifications are associated with anticancer...
| Mechanism | Target | Mechanism | Drug | Clinical trial No. | Phase | Tumor types | Treatment arms | Status |
|-----------|--------|-----------|------|--------------------|-------|-------------|----------------|--------|
| Blockade of alternate coinhibitory immune checkpoint receptors | LAG-3 | LAG-3 fusion protein (IMP321) | Eftilagimod alpha | NCT03625323 | 2 | NSCLC, HNSCC | Eftilagimodalpha + pembrolizumab | Recruiting |
| | IgG4 mAb | Relatlimab (BMS-986016) | NCT02750514 | 2 | NSCLC | Nivolumab ± relatlimab or ipilimumab or BMS-986205 or dasatinib | Active, not recruiting |
| | IgG4 mAb | LAG525 | NCT02460224 | 1,2 | Solid tumor | LAG525 ± spartalizumab (PDR001) | Active, not recruiting |
| | mAb | BI 754111 | NCT03780725 | 1 | NSCLC, HNSCC | BI 754111 + BI 754091 | Active, not recruiting |
| | IgG4 mAb | Mavezelimab (MK-4280) | NCT03516981 | 2 | NSCLC | Pembrolizumab + quavonlimab or MK-4280 or lenvatinib | Completed |
| TIM-3 | Anti-PD-1/TIM-3 bispecific Ab | R07121661 | NCT03708328 | 1 | Solid tumor | R07121661 | Recruiting |
| | Anti-TIM-3 mAb | INCGN02390 | NCT03652077 | 1 | Solid tumor | INCGN02390 | Active, not recruiting |
| | | Sym023 | NCT03489343 | 1 | Solid tumor, lymphoma | Sym023 | Completed |
| | | LY3321367 | NCT03099109 | 1 | Solid tumor | LY330054 (anti-PD-L1), LY3321367, LY330054 + LY3321367 | Active, not recruiting |
| | | Cobolimab (TSR-022) | NCT02817633 | 1 | Solid tumor | Cobolimab ± nivolumab, cobolimab + TSR-042 + TSR-033 or docetaxel | Recruiting |
| | | Sabatolimab (MBG453) | NCT02608268 | 1,2 | Solid tumor | Sabatolimab ± PDR001 or decitabine | Active, not recruiting |
| TiGIT | Anti-TiGIT mAb | Tiagrolumab (MTiG7192/AR-G-6058) | NCT04294810 | 3 | NSCLC | Atezolizumab ± tiagrolumab | Recruiting |
| | | Vibostilimab (MK-7684) | NCT024256421 | 3 | SCLC | Atezolizumab + carboplatin + etoposide ± tiagrolumab | Recruiting |
| | | BMS-986207 | NCT02964013 | 1 | Solid tumor | Vibostilimab ± pembrolizumab ± pemetrexed/carboplatin/vibostilimab ± carboplatin + cisplatin + etoposide | Recruiting |
| | | BMS-986207 | NCT02913313 | 1,2 | Solid tumor | BMS-986207 ± nivolumab | Active, not recruiting |
| BTLA | Anti-OX40 mAb | Domvanalimab (AB-1 54) | NCT04262856 | 2 | NSCLC | Zimberelimab ± domvanalimab ± etrumadenant | Recruiting |
| | Anti-BTLA mAb | Cudarolimab (Bi101) | NCT04672356 | 1 | NSCLC, SCLC | IB939 + sintilimab | Not yet recruiting |
| VISTA | Anti-VISTA mAb | NCT03758001 | 1 | Solid tumor | Cudarolimab ± sintilimab | Recruiting |
| | | NCT04137900 | 1 | Solid tumor | TAB004 | Recruiting |
| | | NCT02671955 | 1 | Solid tumor | JNJ-61610588 | Terminated |
| | | NCT04475523 | 1 | Solid tumor | CI-8993 | Recruiting |
| | Small molecule targeting VISTA and PD-L1 | CA-170 | NCT02812875 | 1 | Solid tumor, lymphoma | CA-170 | Completed |
| Mechanism | Target | Mechanism | Drug | Clinical trial No. | Phase | Tumor types | Treatment arms | Status |
|-----------|--------|-----------|------|--------------------|-------|-------------|----------------|--------|
| Immune stimulatory agents | OX40 Hexavalent OX40 agonist Ab | INBRX-106 | NCT04198766 | 1 | Solid tumor | INBRX-106 + pembrolizumab | Recruiting |
| | PD1-Fc-OX40L | SL-279252 | NCT03894618 | 1 | Solid tumor, lymphoma | SL-279252 | Recruiting |
| | Anti-OX40 agonist mAb | PF-04518600 | NCT021315066 | 1 | Solid cancer | PF-04518600 + PF-05082566 | Completed |
| | Anti-OX40 agonist mAb | INCAGN01949 | NCT02923349 | 1,2 | Solid tumor | INCAGN01949 | Completed |
| | Anti-OX40 agonist mAb | GSK3359609 | NCT03693612 | 2 | Solid tumor | GSK3359609 + tremelimumab, docetaxel + paclitaxel + cetuximab | Recruiting |
| Tumor microenvironment | CSF1R MET, CSF1R, SRC kinase inhibitor | TPX-0022 | NCT03993873 | 1 | Solid tumor | TPX-0022 | Recruiting |
| | CSF1R mAb | Cabiralizumab (FPA008) | NCT02526017 | 1 | Solid tumor | FPA008 + BMS-936558 | Completed |
| | TGF-β TGF-β inhibitor | Galunisertib (LY2157299) | NCT02423343 | 1,2 | Solid tumor | Galunisertib + nivolumab | Completed |
| | TGF-β inhibitor | AVID200 | NCT03834662 | 1 | Solid tumor | AVID200 | Active, not recruiting |
| | TGF-β inhibitor | SAR-439459 | NCT04729725 | 1 | Solid tumor | SAR-439459 + cemiplimab | Not yet recruiting |
| | VEGF VEGFR TKI inhibitor | Vandetanib (ZD6474) | NCT00418886 | 3 | NSCLC | Vandetanib + pemetrexed | Active, not recruiting |
| | Axitinib (AG-013736) | NCT03472560 | 2 | NSCLC, urothelial cancer | Axitinib + avelumab | Active, not recruiting |
| | Apatinib (YN968D1) | NCT03389256 | 2 | NSCLC, urothelial cancer | Apatinib + EGFR-TKI | Not yet recruiting |
| | Anti-VEG mAb | Bevacizumab (L01XC07) | NCT00451906 | 3 | NSCLC | Bevacizumab + first-line chemotherapy | Completed |
| | | IB305 | NCT03802240 | 3 | Non-squamous NSCLC | Nivolumab ± IB305 + pemetrexed + cisplatin | Recruiting |
| | Anti-VEGFR mAb | Ramucirumab (LY3009806) | NCT04340882 | 2 | NSCLC | Ramucirumab + docetaxel + pembrolizumab | Recruiting |
| | Aurora B/VEGFR/ PDGFR/c-Kit/ CSF1R inhibitor | Chiauranib (CS2164) | NCT03216363 | 1 | SCLC | Chiauranib | Recruiting |
| | IL-1β | Ant-IL-1β mAb | Canakinumab (ACZ885) | NCT03626545 | 3 | NSCLC | Canakinumab + docetaxel | Active, not recruiting |
| | IL-6 | IL1RAP Ab | CAN04 | NCT04455214 | 1 | Solid tumor | CAN04 + pembrolizumab | Recruiting |
| | | Anti-IL-6R mAb | Tocilizumab (RO4877533) | NCT04651917 | 1,2 | NSCLC | Tocilizumab + atezolizumab | Not yet recruiting |
| | | Anti-IL-6 mAb | Siltuximab (CNT0 328) | NCT00811911 | 1,2 | Solid tumor | Siltuximab | Completed |
| | IL-8 | Anti-IL-8 mAb | BMS-986253 | NCT04123379 | 2 | NSCLC, HCC | Nivolumab + BMS-81360 or BMS-986253 | Recruiting |
| Mechanism | Target | Drug | Clinical trial No. | Phase | Tumor types | Treatment arms | Status |
|-----------|--------|------|--------------------|-------|-------------|----------------|--------|
| Overcoming Anti–PD-1/PD-L1 Therapy Resistance in NSCLC | | | | | | | |
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immunity, including T cell function, migration, exhaustion, and neoantigen expression (Wang et al., 2020). Epigenetic modifications silence tumor suppressor and apoptosis genes, thereby activating tumor proliferation (Table 1) (Baxter et al., 2014). For instance, the switch/sucrose non-fermentable (SWI/SNF) chromatin remodeling complex decreases the sensitivity of tumor cells to CTLs, leading to a lack of response to immunotherapy (Miao et al., 2018; Pan et al., 2018). Several studies have demonstrated that re-invigoration of exhausted CD8+ T cells and memory T cells is feasible via chromatin remodeling and epigenetic modification (Fig. 1) (Jenkins et al., 2018; Pauken et al., 2016; Ribas et al., 2016).

**POTENTIAL THERAPEUTIC STRATEGIES FOR OVERCOMING ACQUIRED RESISTANCE**

**Clinical trials on the IFN-γ pathway**

Several clinical trials targeting JAK1/2 and STAT are ongoing. In a phase 1/2 study, AZD4205, a JAK1-selective inhibitor, was administered as both monotherapy and combination therapy with osimertinib in advanced NSCLC patients (NCT03450330). A phase 1/2 clinical trial of SC-43, an SHP-1 agonist that inhibits STAT3, in combination with cisplatin therapy for NSCLC is ongoing (NCT04733521).

Activation of stimulator of IFN genes (STING) showed an increase in anti-tumor immunity via the upregulation of proinflammatory chemokines and cytokines, including type I IFNs (Su et al., 2019). STING agonists are a promising option for patients with resistance to immunotherapy. Clinical trials of STING agonists for solid tumors, such as E7766, GSK3745417, and MIW815, are ongoing (NCT04144140, NCT03843359, and NCT03172936, respectively).

**Clinical trials targeting other immune checkpoints**

Randomized, double-blind, and phase 2 clinical trial of anti-TIGIT antibody tiragolumab in combination with atezolizumab (PD-L1 inhibitor) compared with placebo plus atezolizumab in patients with PD-L1-selected NSCLC (CITYSCAPE) revealed improved overall response rates (ORR): 31.3% for tiragolumab group and 16.2% for placebo group) and mean progression-free survival (mPFS: 5.4 months for tiragolumab group and 3.6 months for placebo group) (Rodríguez-Abreu et al., 2020). Other agents targeting immune checkpoint receptors are currently under investigation (Table 2).

**Clinical trials targeting tumor microenvironment**

The A2AR antagonist CPI-444 showed anti-tumor effects as both monotherapy and combination therapy with atezolizumab in patients with anti-PD-1/PD-L1 treatment-refractory renal cell carcinoma (RCC) and NSCLC, with a disease control rate of 36% for monotherapy in NSCLC and 71% for combination therapy in NSCLC (Fong et al., 2017). Other agents targeting the tumor microenvironment, such as CSF1R, TGF-β, VEGF, IL-1/6, A2AR, CD73, IDO1, and B7-H4 inhibitors, are listed in Table 2.

**Clinical trials on epigenetic modification**

Epigenetic modifications include DNA methylation and histone (Kim et al., 2020). DNA methylation is mediated by DNA methyltransferase (DNMT), which regulates silencing of genes and non-coding genomic regions. Histone modification enzymes such as histone methyltransferase (HMT) and histone deacetylase (HDAC) change the structure of chromatin, leading to gene regulation and carcinogenesis (Kanwal and Gupta, 2012). Epigenetic modification enzyme inhibitors such as DNA methyltransferase inhibitors (DNMTis), histone methyltransferase inhibitors (HMTis), and histone deacetylase inhibitors (HDACis) are potential therapeutic targets for immunotherapy resistance (Arenas-Ramirez et al., 2018).

Preclinical studies have shown that both DNMTi and HDACi increase the response to anti-PD-1 therapy in various tumors (Mazzone et al., 2017). One of the histone methyltransferase enzymes, enhancer of zeste homolog 2 (EZH2), is involved in the proliferation, migration, and invasion of various cancer cells such as glioblastoma, ovarian cancer, and prostate cancer (Yamaguchi and Hung, 2014). EZH2 exhibited a silencing effect on antigen presentation and immune reaction, and blocking of EZH2 resulted in synergistic effects with anti-CTLA-4 and IL-2 immunotherapy (Zingg et al., 2017).

For patients with relapsed or refractory malignant mesothelioma, the EZH2 inhibitor tazemetostat was well tolerated and showed a 47% disease control rate in 12 patients (Zauderer et al., 2020). A phase 1/2 clinical trial of tazemetostat monotherapy in patients with advanced solid tumors or B-cell lymphomas is currently underway (NCT01897571). Other clinical trials for epigenetic modulators such as DNMTis, HMTis, HDACis, and adoptive T cell therapy are included in Table 2.

**CONCLUSION**

The advent of immunotherapy has changed the treatment options in NSCLC. Prior to immunotherapy and targeted agents, chemotherapy was the backbone of treatment. Currently, the first-line standard treatment for stage IV NSCLC is anti-PD-1 with or without chemotherapy, with the addition of chemotherapy depending on the PD-L1 expressions of the patients (Mok et al., 2019). There is also the option of anti-PD-L1 and VEGFR inhibitor with chemotherapy in first-line non-squamous NSCLC (Socinska et al., 2018). Recently, front-line nivolumab with ipilimumab in combination with short course chemotherapy showed overall survival benefit in patients with NSCLC and received U.S. Food and Drug Administration approval (Arenas-Ramirez et al., 2018).

Unprecedented results of survival gain in NSCLC have accelerated scientists and clinicians to explore various combinations of immunotherapy with other agents in order to overcome acquired resistance. Indeed, elucidating the mechanisms underlying acquired resistance is necessary to provide treatment options for this subset of patients. Notably, the upregulation IFN-γ pathway, co-inhibition of immune checkpoints such as TIGIT, and inhibition of TGF-β have gained attention as promising potential therapeutic strategies and are awaiting results.

**ACKNOWLEDGMENTS**

This work was supported by National Research Foundation of Korea (NRF) grants funded by the Korean Government (MSIT) (NRF-2017M3A9E9072669, 2017M3A9E8029717,
NRF-2019M3A9B6065231, 2019M3A9B6065221, 2018R1A2A1A05076997, 2017R1A5A1014560).

AUTHOR CONTRIBUTIONS
All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

CONFLICT OF INTEREST
The authors have no potential conflicts of interest to disclose.

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