Molecules and Cells

Minireview

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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Immune checkpoint inhibitors have changed the paradigm of treatment options for non-small cell lung cancer (NSCLC). Monoclonal antibodies targeting programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) have gained wide attention for their application, which has been shown to result in prolonged survival. Nevertheless, only a limited subset of patients show partial or complete response to PD-1 therapy, and patients who show a response eventually develop resistance to immunotherapy. This article aims to provide an overview of the mechanisms of acquired resistance to anti–PD-1/PD-L1 therapy from the perspective of tumor cells and the surrounding microenvironment. In addition, we address the potential therapeutic targets and ongoing clinical trials, focusing mainly on NSCLC.

Keywords: acquired resistance, immune checkpoint inhibitors, non-small cell lung cancer, programmed death-1, programmed death ligand-1

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

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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; Zaretsky 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 (IFNGR1 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 exhaustion, 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 Ig 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, TIGIT, BTLA, VISTA, SIGLEC9)	Blockade of alternate coinhibitory immune checkpoint receptors: LAG-3, TIM-3, TIGIT, BTLA, VISTA, SIGLEC9
		Immune stimulatory agents: OX40, ICOS
Immunosuppressive cells and immunoregulative molecules in tumor microenvironment	Increased immunosuppressive cells (Treg, MDSC, M2 macrophage)	CSF1R inhibitor, TGF-β inhibitor
	Elevated immunosuppressive cytokines (TGF-β, VEGF, IL-6/8)	TGF-β inhibitor, VEGF inhibitor, IL-1β inhibitor, IL-6/8 inhibitor
	Immunoregulative molecules: adenosine pathway, IDO1, B7-H4	A2AR inhibitor/anti-CD73, IDO inhibitor, B7-H4 inhibitor
Epigenetic modification	Tumor suppressor, apoptosis gene modification	Epigenetic modulators: DNMTi, HMTi, HDACi
	Stability of chromatin remodeling complexes	Adoptive T cell therapy

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-lymphocyte 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.

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-

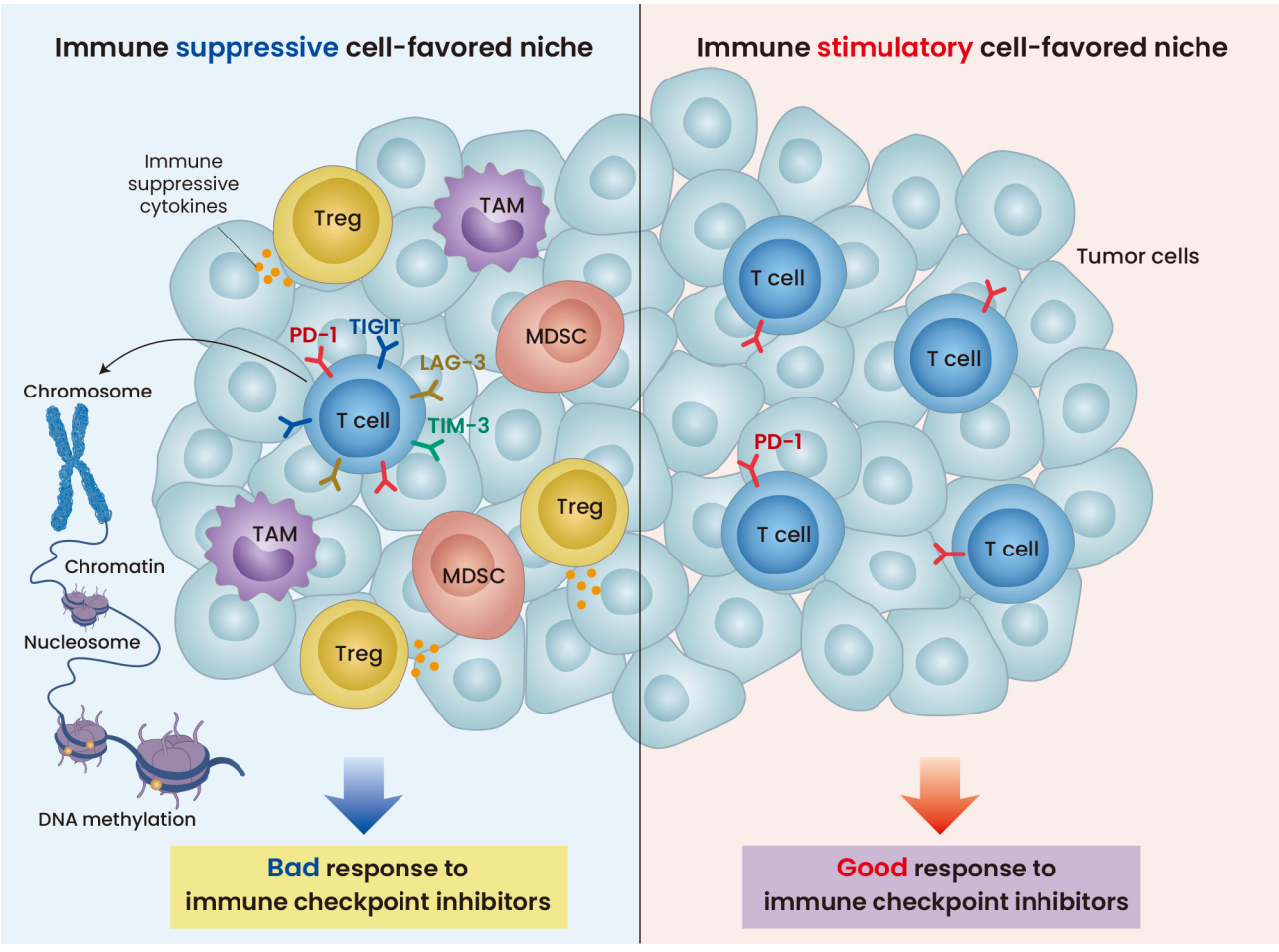

Fig. 1. Immune suppressive and immune stimulatory cell-favored niche. The immune suppressive environment (left) shows the 1) immune suppressive cells including Tregs and MDSCs, 2) the expression of immune suppressive cytokines, and 3) upregulation of immune checkpoint receptors such as TIGIT, LAG-3, 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.

liferation, and survival of the mononuclear phagocyte system and macrophages (Stanley and Chitu, 2014). Blocking CSF1R results in a decrease in tumor-associated microphages, 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 (Garcia-Diaz 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

Table 2. Clinical trials of investigational agents on acquired resistance

Mechanism	Target	Mechanism	Drug	Clinical trial No.	Phase	Tumor types	Treatment arms	Status
INF-γ pathway	STING	STING agonist	E7766	NCT04144140	1	Solid tumor, lymphoma	E7766	Recruiting
			GSK3745417	NCT03843359	1	Solid tumor	GSK3745417 ± pembrolizumab	Recruiting
			MIW815	NCT03172936	1	Solid tumor, lymphoma	MIW815 + PDR001	Active, not recruiting
			SNX281	NCT04609579	1	Solid tumor, lymphoma	SNX281 ± pembrolizumab	Recruiting
			TAK-676	NCT04420884	1	Solid tumor	TAK-676 ± pembrolizumab	Recruiting
	JAK	JAK1 inhibitor	AZD4205	NCT03450330	1,2	NSCLC	AZD4205 + osimertinib	Completed
			Itacitinib (INCB039110)	NCT03425006	2	NSCLC	Itacitinib + pembrolizumab	Active, not recruiting
		JAK1/2 inhibitor	Ruxolitinib (INCB018424)	NCT02145637	1	NSCLC	Ruxolitinib + afatinib	Completed
	STAT	STAT3 inhibitor	TTI-101	NCT03195699	1	Solid tumor	TTI-101	Recruiting
		SHP-1 agonist	SC-43	NCT04733521	1,2	NSCLC, biliary tract cancer	SC-43 + cisplatin	Not yet recruiting

Table 2. Continued

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	Eftilagimod alpha (IMP321)	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	Completed
		IgG4 mAb	Mavezelimab (MK-4280)	NCT03516981	2	NSCLC	Pembrolizumab + quavonlimab or MK-4280 or lenvatinib	Recruiting
	TIM-3	Anti-PD-1/TIM-3 bispecific Ab	RO7121661	NCT03708328	1	Solid tumor	RO7121661	Recruiting
		Anti-TIM-3 mAb	INCAGN02390	NCT03652077	1	Solid tumor	INCAGN02390	Active, not recruiting
			Sym023	NCT03489343	1	Solid tumor, lymphoma	Sym023	Completed
			LY3321367	NCT03099109	1	Solid tumor	LY3300054 (anti-PD-L1), LY3321367, LY3300054 + 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	Tiragolumab (MTIG7192A/RG-6058)	NCT04294810	3	NSCLC	Atezolizumab ± tiragolumab	Recruiting
				NCT04256421	3	SCLC	Atezolizumab + carboplatin + etoposide ± tiragolumab	Recruiting
			Vibostolimab (MK-7684)	NCT02964013	1	Solid tumor	Vibostolimab ± pembrolizumab ± pemetrexed/carboplatin, vibostolimab + carboplatin + cisplatin + etoposide	Recruiting
			BMS-986207	NCT02913313	1,2	Solid tumor	BMS-986207 ± nivolumab	Active, not recruiting
			Domvanalimab (AB-154)	NCT04262856	2	NSCLC	Zimberelimab ± dombvanalimab ± etrumadenant	Recruiting
			IBI939	NCT04672356	1	NSCLC, SCLC	IBI939 + sintilimab	Not yet recruiting
	BTLA	Anti-OX40 mAb	Cudarolimab (IBI101)	NCT03758001	1	Solid tumor	Cudarolimab ± sintilimab	Recruiting
		Anti-BTLA mAb	TAB004	NCT04137900	1	Solid tumor	TAB004	Recruiting
	VISTA	Anti-VISTA mAb	JNJ-61610588	NCT02671955	1	Solid tumor	JNJ-61610588	Terminated
			CI-8993	NCT04475523	1	Solid tumor	CI-8993	Recruiting
		Small molecule targeting VISTA and PD-L1	CA-170	NCT02812875	1	Solid tumor, lymphoma	CA-170	Completed

Table 2. Continued

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	NCT02315066	1	Solid cancer	PF-04518600 ± PF-05082566	Completed
			INCAGN01949	NCT02923349	1,2	Solid tumor	INCAGN01949	Completed
	ICOS	Anti-ICOS mAb	GSK3359609	NCT03693612	2	Solid tumor	GSK3359609 + tremelimumab, docetaxel + paclitaxel + cetuximab	Recruiting
			JTX-2011	NCT02904226	1,2	Solid tumor	JTX-2011 + pembrolizumab or nivolumab or ipilimumab	Completed
			KY1044	NCT03829501	1,2	Solid tumor	KY1044 ± atezolizumab	Completed
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-βR inhibitor	Galunisertib (LY2157299)	NCT02423343	1,2	Solid tumor	Galunisertib + nivolumab	Completed
			TEW 7197	NCT02160106	1	Solid tumor	TEW-7197	Completed
		TGF-β inhibitor	AVID200	NCT03834662	1	Solid tumor	AVID200	Active, not recruiting
		Anti-TGF-βmAb	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	EGFR T790M-negative NSCLC	Apatinib + EGFR-TKI	Not yet recruiting
		Anti-VEGF mAb	Bevacizumab (L01XC07)	NCT00451906	3	NSCLC	Bevacizumab + first-line chemotherapy	Completed
			IBI305	NCT03802240	3	Non-squamous NSCLC	Sintilimab ± IBI305 + 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)	NCT03216343	1	SCLC	Chiauranib	Recruiting
	IL-1β	Anti-IL-1β mAb	Canakinumab (ACZ885)	NCT03626545	3	NSCLC	Canakinumab + docetaxel	Active, not recruiting
		IL1RAP Ab	CAN04	NCT04452214	1	Solid tumor	CAN04 + pembrolizumab	Recruiting
	IL-6	Anti-IL-6R mAb	Tocilizumab (RO4877533)	NCT04691817	1,2	NSCLC	Tocilizumab + atezolizumab	Not yet recruiting
		Anti-IL-6 mAb	Siltuximab (CNTO 328)	NCT00841191	1,2	Solid tumor	Siltuximab	Completed
	IL-8	Anti-IL-8 mAb	BMS-986253	NCT04123379	2	NSCLC, HCC	Nivolumab + BMS-813160 or BMS-986253	Recruiting

Table 2. Continued

Mechanism	Target	Mechanism	Drug	Clinical trial No.	Phase	Tumor types	Treatment arms	Status
Tumor microenvironment	A2AR	A2AR antagonist	PBF-509	NCT02403193	1,2	NSCLC	PBF-509 + PDR001	Active, not recruiting
			Etrumadenant (AB928)	NCT03846310	1	Lung cancer	Etrumadenant + carboplatin + pemetrexed	Recruiting
	CD73	Small molecule CD73 inhibitor	LY3475070	NCT04148937	1	Solid tumor	LY3475070 ± pembrolizumab	Recruiting
		Anti-CD73 mAb	CPI-006	NCT03454451	1	Solid cancer, NHL	CPI-006 ± ciforadenant or pembrolizumab	Recruiting
			Oleclumab (MEDI9447)	NCT03381274	1,2	EGFR-mutant NSCLC	MEDI9447 + osimertinib or AZD4635	Active, not recruiting
			Sym024	NCT04672434	1	Solid tumor	Sym021 ± Sym024	Recruiting
			NZV930	NCT03549000	1	Solid tumor	NZV930 ± PDR001 ± NIR178	Recruiting
	IDO1	IDO inhibitor	Indoximod (NLG-8189)	NCT02460367	1	NSCLC	Indoximod + docetaxel + tergenpumatucel-L	Active, not recruiting
	B7-H4	B7-H4 Ab	FPA150	NCT03514121	1	Solid tumor	FPA150 + pembrolizumab	Active, not recruiting
Epigenetic modulators	Hypomethylating agents	DNMTi	Guadecitabine (SGI-110)	NCT03913455	2	SCLC	Guadecitabine + carboplatin	Active, not recruiting
		Cytidine nucleoside analogue	CC-486	NCT02546986	2	NSCLC	Pembrolizumab ± CC-486	Active, not recruiting
		Deoxycitidine analogue	Aza-TdCyd	NCT03366116	1	Solid tumor	Aza-TdCyd	Recruiting
	HMTi	EZH2 inhibitor	Tazemetostat (EPZ-6438)	NCT01897571	1,2	Solid tumor, B-cell lymphoma	Tazemetostat	Active, not recruiting
	HDACi	HDAC inhibitor	ACY-241	NCT02635061	1	NSCLC	ACY-241 + nivolumab	Active, not recruiting
			Mocetinostat (MGCD01013)	NCT02954991	2	NSCLC	Nivolumab + glesatinib or sitravatinib or mocetinostat	Active, not recruiting
			Entinostat (SNDX-275)	NCT01928576	2	NSCLC	Entinostat + azacitidine + nivolumab	Recruiting
			Vorinostat (MK0683)	NCT02638090	1,2	NSCLC	Pembrolizumab ± vorinostat	Recruiting
			Abexinostat (PCI-24781)	NCT03590054	1	Solid tumor	Abexinostat + pembrolizumab	Recruiting
	Adoptive T cell therapy		Genetically modified T cells	NCT02408016	1,2	NSCLC, mesothelioma	Autologous WT1-TCRc4 Gene-transduced CD8-positive Tcm/Tn Lymphocytes	Active, not recruiting
			Genetically modified T cells	NCT02706392	1	ROR1-positive Solid tumor	ROR1 CAR-specific autologous T-Lymphocytes	Recruiting

STING, stimulator of IFN genes; JAK, Janus kinase; NSCLC, non-small cell lung cancer; STAT, signal transducer and activators of transcription; SHP-1, Src-homology2 domain-containing phosphatase-1; LAG-3, lymphocyte-associated gene 3; HNSCC, head and neck squamous cell carcinoma; TIM-3, T-cell immunoglobulin and mucin domain-3; anti-PD-L1, anti-programmed cell death ligand 1; TIGIT, T-cell immunoglobulin and ITIM domain; SCLC, small cell lung cancer; BTLA, B and T-lymphocyte attenuator; VISTA, V-domain immunoglobulin suppressor of T-cell activation; ICOS, inducible T-cell costimulator; CSF1R, colony stimulating factor 1 receptor; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor; IL, interleukin; Anti-IL-6R-mAb, anti-interleukin-6 receptor monoclonal antibody; TKI, tyrosine kinase inhibitor; EGFR, epidermal growth factor receptor; HCC, hepatocellular carcinoma; A2AR, adenosine A2A receptor; NHL, non-Hodgkin lymphoma; IDO, indoleamine 2,3-dioxygenase; DNMTi, indoleamine 2,3-dioxygenase; HMTi, histone methyltransferase inhibitor; HDACi, histone deacetylase inhibitor; ROR1, tyrosine-protein kinase transmembrane receptor.

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 improvement in 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) (Rodriguez-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 (Socinski 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.

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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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