



REVIEW ARTICLE

Crosstalk between the B7/CD28 and EGFR pathways: Mechanisms and therapeutic opportunities

Xiaoxin Ren ^{a,1}, Yixian Li ^{b,1}, Christopher Nishimura ^a,
Xingxing Zang ^{a,c,d,*}

^a Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY 10461, USA

^b Division of Pediatric Hematology/Oncology/Transplant and Cellular Therapy, Children's Hospital at Montefiore, Bronx, NY 10467, USA

^c Department of Medicine, Albert Einstein College of Medicine, New York, NY 10461, USA

^d Department of Urology, Albert Einstein College of Medicine, New York, NY 10461, USA

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Abstract Somatic activating mutations in the epidermal growth factor receptor (EGFR) are one of the most common oncogenic drivers in cancers such as non-small-cell lung cancer (NSCLC), metastatic colorectal cancer, glioblastoma, head and neck cancer, pancreatic cancer, and breast cancer. Molecular-targeted agents against EGFR signaling pathways have shown robust clinical efficacy, but patients inevitably experience acquired resistance. Although immune checkpoint inhibitors (ICIs) targeting PD-1/PD-L1 have exhibited durable anti-tumor responses in a subset of patients across multiple cancer types, their efficacy is limited in cancers harboring activating gene alterations of EGFR. Increasing studies have demonstrated that up-regulation of new B7/CD28 family members such as B7-H3, B7x and HHLA2, is associated with EGFR signaling and may contribute to resistance to EGFR-targeted therapies by creating an immunosuppressive tumor microenvironment (TME). In this review, we discuss the regulatory effect of EGFR signaling on the PD-1/PD-L1 pathway and new B7/CD28 family member pathways. Understanding these interactions may inform combination therapeutic strategies and potentially overcome the current challenge of resistance to EGFR-targeted therapies. We also summarize clinical data of anti-PD-1/PD-L1 therapies in EGFR-mutated cancers, as well as

* Corresponding author. Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY 10461, USA.
E-mail address: xingxing.zang@einsteinmed.org (X. Zang).

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¹ These authors contributed equally to this study.

ongoing clinical trials of combination of EGFR-targeted therapies and anti-PD-1/PD-L1 immunotherapies.

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Introduction

The epidermal growth factor receptor (EGFR, also known as ERBB1 or HER1) belongs to the ERBB family of cell-surface receptor tyrosine kinases (RTKs) that also includes HER2 (ERBB2), HER3 (ERBB3) and HER4 (ERBB4).^{1,2} EGFR ligands binding to EGFR triggers homodimerization or heterodimerization with other ERBB members and induces receptor phosphorylation and activation of downstream signaling cascades including the RAS/RAF/MEK/ERK/MAPK, PI3K/AKT/mTOR, and JAK/STAT signaling pathways, leading to cell proliferation, evasion of apoptosis, angiogenesis, and metastasis.^{1,3,4} EGFR is frequently expressed in epithelial tumors such as non-small cell lung cancer (NSCLC), breast, colorectal, pancreatic, and head and neck squamous cell carcinoma (HNSCC).^{3,5} Somatic EGFR-activating mutations, most commonly exon 19 deletions (EGFR^{Δ19}) and L858R, account for 85% of all EGFR mutations,^{6–8} and promote oncogenesis, tumor growth, and progression.^{5,9} Molecular-targeted therapies, including tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (mAbs), against EGFR signaling have been successfully developed and are now the first-line treatment for EGFR-mutant NSCLC, resulting in the improved survival of patients with cancers harboring these alterations.^{4,10,11} However, cancer patients who initially benefit from EGFR-targeted therapies inevitably develop acquired resistance. A better understanding of the complex mechanisms of resistance may provide an opportunity to develop new mechanism-based inhibitors and combination therapies to prevent or overcome therapeutic resistance in tumors.

The T cell-based immune system has evolved to recognize and destroy abnormal cells, such as pathogen-infected cells and cancer cells, through interaction of the T cell receptor (TCR) on T cells with peptide-major histocompatibility complexes (MHC) on target cells.^{12,13} The functional activity of antigen-specific T cells is to a very large extent regulated by a series of co-stimulatory receptors, and co-inhibitory receptors (also known as immune checkpoints), and their ligands, thus playing a critical role in maintaining self-tolerance and limiting tissue damages.¹³ Among all immune checkpoints, the B7-1/B7-2–CTLA-4 and PD-L1–PD-1 pathways of the B7/CD28 family have stood out because of their proven value as therapeutic targets in a large number of malignancies. The ability to successfully target checkpoint regulators has led to growing numbers of clinical trials with antibodies targeting the pathways of other B7/CD28 members. The current B7 family members and their receptors can be phylogenetically divided into three groups: group I consisting of B7-1/B7-2/CD28/CTLA-4 and B7h (ICOSL)/ICOS; group II containing PD-L1/PD-L2/PD-1; group III including B7-H3 (CD276), B7x (B7-H4/B751), and HHLA2

(B7γ/B7H7/B7-H5)/TMIGD2 (IGPR-1/CD28H)/KIR3DL3.^{14–20} Compelling evidence indicates that B7 molecules not only provide crucial positive signals to stimulate and support T cell activation, but also offer negative signals that control and suppress T-cell responses.

Increasing numbers of studies have demonstrated that the upregulation of B7/CD28 family members, such as PD-L1, B7x and HHLA2, is associated with activating EGFR mutations,^{21–28} which may contribute to resistance to EGFR-targeted therapies by creating an immunosuppressive tumor microenvironment (TME). In this review, we will discuss the regulatory effect of EGFR signaling on the B7/CD28 pathways, which may inform future combination therapeutic strategies and overcome the current challenge of EGFR-TKI resistance. We also summarize clinical data and ongoing clinical trials of combination of EGFR-targeted therapies with anti-PD-1/PD-L1 immunotherapies in EGFR-mutant cancers.

Crosstalk between the EGFR pathway and the PD-1/PD-L1 axis

The role of the EGFR signaling pathway on the PD-1/PD-L1 pathway

The PD-1/PD-L1 axis is a critical co-inhibitory immune pathway involved in the tumor immune evasion by counteracting T cell effector functions and promoting exhaustion. Aberrant PD-L1 expression is frequently observed in human cancers. PD-L1 binding to its receptor PD-1 on activated T cells alters the function of T cells by means of inhibiting T cell proliferation and survival, cytokine production, and other effector functions. Consistent with the role of PD-1/PD-L1 in suppressing T cell functions, antibody-based PD-1/PD-L1 inhibitors display striking responses in patients with diverse advanced cancers, which has led to clinical approval for the treatment of melanoma, NSCLC, renal cell carcinoma (RCC), Hodgkin's lymphoma, bladder cancer, HNSCC, Merkel-cell carcinoma, and microsatellite instable-high (MSI-H) or mismatch repair-deficient (dMMR) solid tumors.^{29,30} Despite considerable improvement in patient outcomes, durable responses to these therapies are observed only in a minority of patients. Indeed, PD-L1 expression on tumor cells and in TME has been closely associated with clinical responses,^{31–34} highlighting the necessity of a better understanding of the mechanisms which control PD-L1 expression.

Expression of PD-L1 in tumor cells is determined by complex regulatory mechanisms, including but not limited to, aberrant oncogenic signaling, inflammatory signaling,

posttranslational modulation, and genomic alterations.³⁰ Compelling findings have demonstrated that aberrant oncogenic signaling may contribute to tumor outgrowth by driving PD-L1 expression in a tumor cell-intrinsic manner.³⁰ The EGFR signaling pathway is one of the most important oncogenic pathways in NSCLC,^{35–37} metastatic colorectal cancer (CRC),^{38,39} glioblastoma,^{40–42} HNSCC,⁴³ pancreatic cancer,^{44,45} breast cancer,^{46–48} and many other types of tumors. A series of preclinical and retrospective studies show that activating EGFR mutations as well as stimulation with the ligands can induce PD-L1 expression in bronchial epithelial cells,⁴⁹ NSCLC,^{21,50} HNSCC,⁵¹ and breast cancer⁵² cells via downstream effector pathways. In line with this finding, the expression level of PD-L1 is significantly higher in NSCLC cell lines with mutant EGFR than in cells with wild type (WT) EGFR.⁴⁹ Pharmacological inhibition of EGFR activity by EGFR-TKIs, e.g., gefitinib or erlotinib, decreases the constitutive expression of PD-L1 in NSCLC with mutant EGFR.^{21,49,50,53,54} In an EGFR-driven mouse model of lung cancer, microarray expression profiling of mice carrying EGFR mutations as compared to WT controls reveals elevated PD-L1 and PD-1 expression, thus leading to an immunosuppressive lung microenvironment.⁴⁹

Mechanisms underlying the EGFR-mediated upregulation of PD-L1 are complicated. Upregulation of PD-L1 expression in EGFR mutant NSCLC cells can be blocked by the mTOR inhibitor rapamycin⁵⁵ and by an ERK inhibitor,²¹ suggesting mTOR and ERK pathways may be responsible for the EGFR-mediated PD-L1 expression. In addition, several studies in HNSCC and NSCLC provide evidence for a model in which NF- κ B, STAT3, and/or JAK2-STAT1 may also serve as mediators between EGFR signaling and PD-L1 expression^{50,51,54} (Fig. 1).

The effectiveness of PD-1/PD-L1 inhibitors in EGFR mutant NSCLC is currently controversial. Theoretically, increased PD-L1 expression in EGFR mutant and EGFR-TKI resistant NSCLC cell lines suggests that PD-1/PD-L1 blockade may be a promising approach in NSCLC with EGFR mutations. However, the overall clinical proof of principle currently could not fully support this therapeutic approach in EGFR-mutant NSCLC patients. Patients with NSCLC harboring EGFR mutations have poor clinical outcomes and are associated with low objective response rate (ORR) in response to treatment with PD-1/PD-L1 inhibitors.^{56–58} In a retrospective analysis of 58 patients treated with PD-1/PD-L1 inhibitors, relatively higher ORRs were observed in EGFR-WT patients (23.3%) versus EGFR-mutant patients (3.6%).⁵⁶ Several meta-analyses exploring the efficacy of PD-1/PD-L1 inhibitors versus the chemotherapy docetaxel show that PD-1/PD-L1 inhibitors prolonged the overall survival (OS) of patients with EGFR-WT, but not EGFR-mutant advanced NSCLC.^{58–61} In addition, one study not only analyzed the data from NSCLC, but also other types of cancer, and demonstrated that significantly prolonged progression-free survival (PFS) and OS were observed in patients with EGFR-WT cancers receiving PD-1 blockade but not in those with EGFR-mutated cancers.⁶² The underlying association between EGFR mutations and immune resistance remains largely unclear. We will review the potential mechanisms by which EGFR mutations impair response to PD-1/PD-L1 blockade and summarize the

current clinical data of ICIs in EGFR-mutant cancers in the following section (Section [Aberrant EGFR signaling regulates anti-tumor immunity and is associated with resistance to ICIs](#)).

The PD-1/PD-L1 pathway modulates the efficacy of EGFR-TKIs

EGFR TKIs have been a first-line therapy in the treatment of NSCLC harboring EGFR sensitive mutations. Four FDA-approved EGFR TKIs are currently in clinical use, with response rates of ~50–80%, including the first-generation non-covalent inhibitors erlotinib and gefitinib, the second-generation covalent inhibitor afatinib, and the more recently approved third-generation, WT-sparing, mutant EGFR-specific TKI osimertinib.^{63–65} Growing evidence suggests that inhibiting EGFR by EGFR-TKIs modulates the tumor immune microenvironment, including enhancing MHC I and MHC II expression, attenuating the suppressive function of regulatory T cells (Tregs), promoting the activity of cytotoxic T cells (CTLs) and reducing the apoptosis of T cells.^{21,66–69} Although treatment with EGFR TKIs improves outcomes in patients with NSCLC, responses to these agents remain largely incomplete and temporary due to acquired resistance.

The association between PD-L1 expression status and clinical outcomes of EGFR-TKIs in patients with EGFR mutant advanced NSCLC has been explored. However, currently there is no consensus on the prognostic role of PD-L1 expression in EGFR mutant NSCLC treated with EGFR-TKIs. Two studies have reported that PD-L1 positive compared to PD-L1 negative EGFR-mutant NSCLC patients treated with EGFR-TKIs have a significantly longer PFS.^{70,71} Conversely, several other studies show that high tumor PD-L1 expression in EGFR-mutant NSCLC was associated with a shorter PFS.^{72,73} The inconsistent findings among the studies may be due to differences in prior treatment, detection approach, PD-L1 antibody clones, and scoring cutoffs. PD-L1 expression is dynamic during the time course of EGFR-TKI treatment. The expression of PD-L1 could be reduced by EGFR-TKIs,^{21,49,54} while it is upregulated in some patients who acquired resistance to EGFR-TKIs.^{56,74,75} The mechanisms underlying acquired resistance to EGFR-TKIs are complex, and more than 50% were mediated by T790M.^{76,77} The third-generation EGFR-TKI osimertinib significantly improves survival of T790M mutation positive patients, but not T790M mutation negative patients who developed resistance to EGFR-TKIs. Studies show PD-L1 expression was significantly higher in T790M negative patients compared to T790M positive patients.⁷⁴ Collectively, PD-L1 upregulation might be correlated with EGFR-TKI resistance in a subset of patients, especially in T790M negative patients.

Crosstalk between the EGFR pathway and new B7 family pathways

The molecules B7-H3, B7x and HHLA2 in the third group of B7/CD28 family have been found to be overexpressed in various human malignancies.^{78,79} Cumulative studies reveal

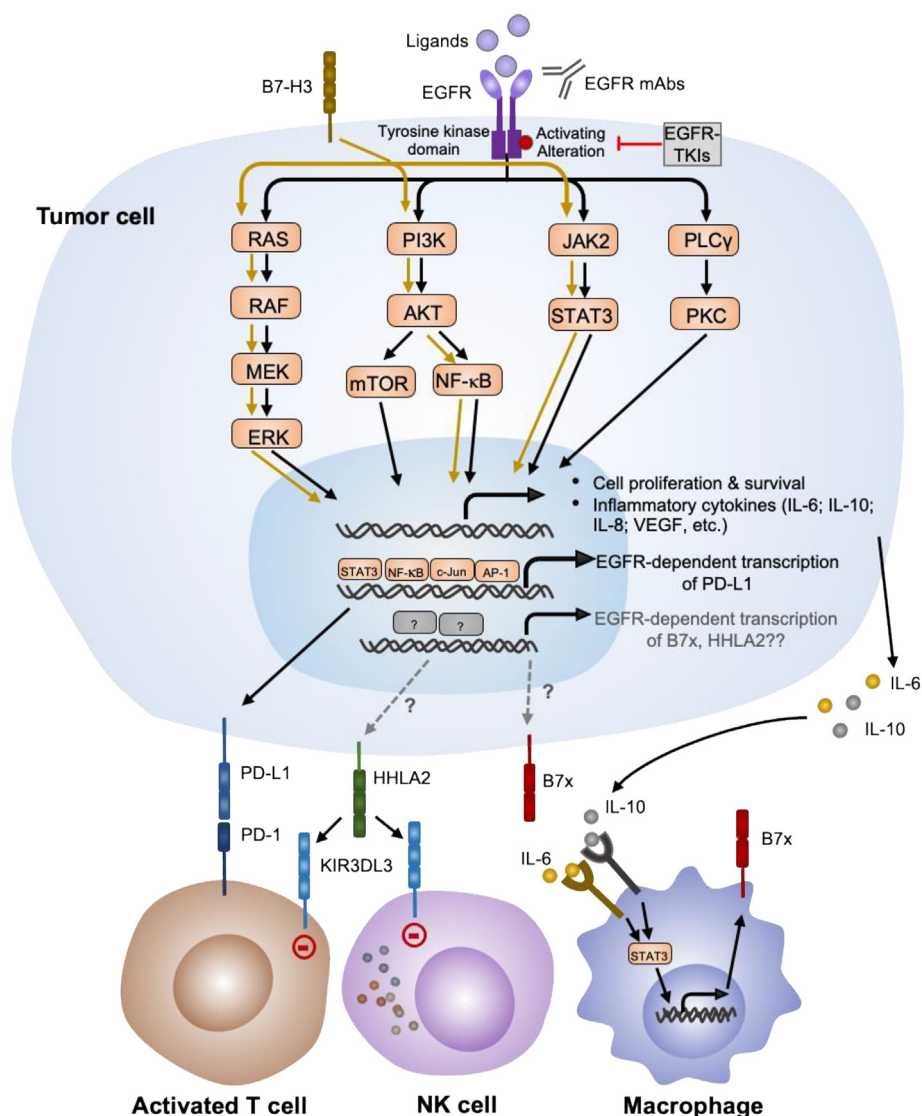


Figure 1 Schematic overview of crosstalk between the EGFR signaling and the B7/CD28 family. Pathways known to be involved in the EGFR-mediated signaling are depicted. Activation of EGFR by either ligand interaction or activating gene alteration leads to downstream signaling pathways that ultimately drive tumor proliferation, survival and induce pro-tumoral cytokines. EGFR signaling activation induces PD-L1 upregulation by activation of the PI3K/AKT, RAS/RAF/MEK/ERK or JAK/STAT3 pathways. These pathways involved in the EGFR signaling also mediate B7-H3 signaling, however, the crosstalk between these two pathways remains to be determined. B7x and HHLA2 are also associated with activating EGFR mutations through unclear mechanisms. IL-6 and IL-10 induced by EGFR activation may induce B7x expression in macrophages through JAK/STAT3 signaling, which represents a potential mechanism by which EGFR upregulates B7x expression. The upregulation of HHLA2 could result in the inhibition of T and NK cell response through interacting with its inhibitory receptor KIR3DL3. JAK, Janus kinase; NF- κ B, nuclear factor kappa B; PI3K, phosphatidylinositol-4,5-Bisphosphate 3-kinase; PLC, phospholipase C; PD-1, programmed cell death protein-1; PD-L1, programmed death ligand-1; STAT3, signal transducer and activator of transcription 3; HHLA2, human endogenous retrovirus-H long terminal repeat-associating protein 2; KIR3DL3, killer cell immunoglobulin-like receptor, three immunoglobulin domains and long cytoplasmic tail 3; NK cell, natural killer cell; B7x, B7 homolog x.

their aberrant expression is associated with poor prognoses and clinical outcomes in certain human cancers. Given their emerging roles in suppressing anti-tumor immune responses, there are gaining increasing interest as new therapeutic targets for the treatment of human diseases. In this section, we will review the advances of B7-H3, B7x and HHLA2 and their association with the EGFR pathway, thereby expanding the scope of treatment strategies for

cancer patients with EGFR mutations and acquired EGFR-TKI resistance.

The correlation of B7-H3 with the EGFR signaling pathway

B7-H3 (also known as CD276) is a type I transmembrane protein that belongs to the B7 family. B7-H3 expression is

not detectable on peripheral blood lymphocytes, but is induced on T cells, B cells and NK cells upon *in vitro* stimulation. B7-H3 was initially identified as a T cell costimulator in humans due to its ability to promote T-cell proliferation and interferon gamma (IFN- γ) secretion.⁸⁰ However, increasing evidence suggests that it is a negative regulator.^{81–84} In mice, B7-H3 inhibits T cell activation and effector cytokine production, so an anti-B7-H3 antagonistic mAb exacerbated experimental autoimmune encephalomyelitis (EAE) *in vivo*.⁸⁵ In the context of graft-versus-host disease (GVHD), absent B7-H3 expression on allogeneic donor T cells leads to accelerated GVHD lethality associated with increased T-cell proliferation and inflammatory cytokines,⁸⁴ demonstrating that B7-H3 provides a negative signal to T cells. In humans, B7-H3 expression is absent or low in normal tissues, however, more than 60% and up to 93% of patient tumor tissues display aberrant expression of B7-H3 in the vast majority of malignancies, and its expression is significantly linked to poor prognosis and decreased OS in patients with RCC,⁸⁶ lung cancer,⁸⁷ prostate cancer,⁸⁸ CRC,⁸⁹ gallbladder cancer,⁹⁰ esophageal squamous cancer,⁹¹ osteosarcoma,⁹² and breast cancer,⁹³ suggesting that B7-H3 is an attractive biomarker and high-value target for multiple cancer immunotherapy strategies.

B7-H3 promotes cancer cell survival, invasion, migration, metastasis and drug resistance in various types of cancer that is independent of its immune function by acting upstream from signal transduction pathways, such as PI3K/AKT, JAK2/STAT3, and Raf/MEK/ERK1/2, to induce anti-apoptotic and proliferative mechanisms.^{94–98} These pathways are also involved in EGFR-triggered signaling in lung adenocarcinoma cells (Fig. 1), thus the correlation between the two signaling pathways has been elucidated in several studies. B7-H3 knockout increases susceptibility of NSCLC cell lines harboring activating EGFR mutations to EGFR inhibitor gefitinib.²⁵ Furthermore, B7-H3 ablation displayed significant synergistic effects with gefitinib *in vitro*.²⁵ Additionally, high B7-H3 expression is associated with EGFR WT status (multivariable OR = 2.80, 95% CI = 1.38–5.84; $P = 0.0042$),⁹⁹ hinting at the potential effectiveness of anti-B7-H3 therapy in EGFR WT lung adenocarcinoma. In pancreatic cancer, activation of B7-H3 by an agonist induced the expression of EGFR,²⁴ suggesting that B7-H3 may be involved in regulating the EGFR signaling pathway in different cancer settings. However, the exact mechanism needs to be explored. Collectively, these studies reveal the correlation of B7-H3-induced signaling with the EGFR signaling pathway, and the translational potential of combined therapy targeting B7-H3 and EGFR in human cancers.

B7x is aberrantly expressed in EGFR mutated cancers

B7x (also known as B7S1, B7-H4, VTCN1) is a B7 ligand that was discovered by us and others simultaneously.^{14,100,101} B7x mRNA is widely distributed in mouse and human peripheral tissues, however, protein expression is restricted and can be induced on antigen presenting cells (APCs) after *in vitro* stimulation.^{14,102} Previous studies have found that aberrant B7x is expressed on a broad spectrum of cancers, including those of the stomach, kidney, ovary, lung, uterus,

breast, prostate, and others.⁷⁹ The binding of B7x to its unidentified receptor(s) inhibits TCR-mediated T cell proliferation by cell-cycle arrest and decreases IL-2 production,^{14,100,101} indicative of its role as a negative regulator of T cell responses. In addition to dampening effector T cell activity, B7x also promotes myeloid-derived suppressor cells^{103–106} and macrophages,^{107,108} thus inducing an overall immunologically tolerant and immunosuppressive TME. Clinically, B7x expression is significantly associated with greater disease progression and poorer prognosis in patients with RCC,^{106,109} lung cancer,^{110,111} CRC,¹¹² cholangiocarcinoma,¹¹³ glioma,¹⁰⁸ pancreatic cancer,¹¹⁴ and others. Interestingly, the role of B7x in cancer seems to be conserved across mammalian species given that B7x expression is also associated with worse prognosis in canine bladder cancer.¹¹⁵ Blockade of B7x has demonstrated significant therapeutic efficacy across multiple murine models by reducing primary tumor growth and metastasis.^{116–119} Collectively, these findings recognize that targeting B7x is a very attractive strategy for cancer immunotherapy.

Our previous study shows that, in tissue microarrays consisting of 392 resected NSCLC tumors, B7x was expressed in 69% of tumors and the co-expression of PD-L1 with B7x was infrequent.²⁷ Although our univariate analysis revealed no significant difference between WT and mutated EGFR tumors in both the discovery ($P = 0.06$) and validation cohorts ($P = 0.55$),²⁷ another independent study showed that the expression of B7x in the EGFR mutant group was significantly higher than those in the WT group (mutant vs. WT IHC score: 3.250 [0–7.000] vs. 5.000 [1.000–7.000]; $P = 0.045$).²⁸ Moreover, nearly half of the PD-L1 negative patients expressed B7x, indicating that B7x may be a promising immune target for patients with EGFR mutant lung cancer independent of PD-L1 expression. The functional mechanisms of upregulation of B7x in EGFR mutant cancers have not yet been investigated. Previous findings show that EGFR-driven lung tumors inhibit anti-tumor immunity by activating the PD-1/PD-L1 pathway to suppress T-cell function and increase levels of proinflammatory cytokines including IL-6, IL-10, IL-8, and VEGF.⁴⁹ Of note, IL-6, together with IL-10, can induce B7x expression in infiltrating myeloid cells through JAK/STAT3 signaling,^{108,120} which may represent a potential mechanism by which EGFR upregulates B7x expression in NSCLC (Fig. 1).

HHLA2 is associated with EGFR mutation in NSCLC

We recently identified HHLA2 (also known as B7-H7, B7-H5, B7y) as the newest member of the B7 family¹⁵ that shares 23%–33% similarity to other human B7 proteins, with the greatest phylogenetical similarity to B7-H3 and B7x. Notably, HHLA2 is the only B7 family member that is found in humans but not in mice. The HHLA2 protein is constitutively expressed on human monocytes/macrophages, and its expression on mature DCs and monocytes is modestly upregulated by inflammatory signals like lipopolysaccharides (LPS), IFN- γ , and poly I:C.^{15,16} HHLA2 protein is widely expressed in human cancers from the breast, lung, thyroid, melanoma, pancreas, ovary, liver, bladder, colon, prostate, kidney, and esophagus.¹⁷ Studies have shown that high

expression of HHLA2 in tumors is correlated with a worse prognosis for lung cancer, osteosarcoma,¹²¹ gastric cancer,¹²² breast carcinoma,¹⁷ ccRCC,¹²³ intrahepatic cholangiocarcinoma,¹²⁴ bladder urothelial carcinoma¹²⁵ and CRC,¹²⁶ but is associated with a better prognosis for pancreatic ductal adenocarcinoma¹²⁷ and glioma.¹²⁸ This could be because HHLA2 can exert either a costimulatory or coinhibitory effect on T and NK cell activation. The costimulatory effect of HHLA2 is mediated through the CD28 family member transmembrane and immunoglobulin domain containing 2 (TMIGD2, also called CD28H or IGPR-1).^{16,17} Recently, we and other groups identified killer cell immunoglobulin-like receptor, three immunoglobulin domains and long cytoplasmic tail 3 (KIR3DL3) as an inhibitory receptor for HHLA2 on T and NK cells.^{19,20,129,130} Monoclonal antibodies targeting KIR3DL3 and HHLA2 effectively blocked both receptor–ligand interactions and inhibitory functions.¹⁹ HHLA2 expression is nonoverlapping with PD-L1 expression in NSCLC²⁷ and intrahepatic cholangiocarcinoma,¹²⁴ suggesting that HHLA2 mediates a mechanism of tumor immune evasion independent from the PD-1/PD-L1 pathway. Given its emerging roles in suppressing tumor immune responses with different immune evasion mechanisms from the PD-1/PD-L1 pathways, HHLA2 is attracting interest as a new therapeutic immune target.

We previously investigated the expression and clinical significance of HHLA2 in human lung cancer and found that HHLA2 was not detected in most of normal lung tissues but expressed in 66% of 679 NSCLC tumor tissues across different subtypes.²⁷ EGFR-mutated NSCLC was significantly associated with higher tumor HHLA2 expression in both the discovery (EGFR vs. WT: 76% vs. 53%, $P = 0.01$) and validation cohorts (89% vs. 69%, $P = 0.01$).²⁶ In line with our findings, another independent study also observed HHLA2 was prominently upregulated in lung adenocarcinoma with EGFR mutation.²⁸ In the multivariate analysis, high tumor-infiltrating lymphocyte intensity and EGFR mutation status were independently associated with HHLA2 expression in lung adenocarcinoma.²⁶ In addition, in the EGFR-mutated subgroup, patients with high HHLA2⁺ tumors trended toward poorer survival,²⁶ suggesting HHLA2 may be a new therapeutic target for lung cancer, especially for patients with EGFR mutations. Interestingly, a notably negative correlation between WT EGFR and HHLA2 expression was demonstrated in a study including 47 NSCLC cancer tissues and 4 cell lines.¹³¹ EGFR silencing and EGFR-TKI treatment significantly increased HHLA2 mRNA and protein expression in NCI-H1299 cells.¹³¹ Collectively, these studies suggest that EGFR may participate in immune evasion through the regulation of HHLA2 expression in NSCLCs. Further investigation on the mechanism(s) regulating HHLA2 expression in EGFR-mutated cancers is clearly warranted.

Aberrant EGFR signaling regulates anti-tumor immunity and is associated with resistance to ICIs

ICIs, represented by anti-PD-1/PD-L1 immunotherapies, have showed superior outcomes over traditional chemotherapy in the treatment for multiple solid tumors.

However, they failed to achieve similar efficacy in EGFR-mutant NSCLCs in multiple large-scale, randomized controlled clinical trials.^{31,132,133} A phase II clinical trial was designed to study the efficacy of the anti-PD-1 antibody pembrolizumab in patients with EGFR-mutant, PD-L1 positive, TKI treatment naïve, advanced NSCLC, but it was terminated early due to lack of efficacy after 11/25 planned patients were treated.¹³⁴ This result was validated by a systemic review and meta-analysis studying patients with advanced NSCLC treated with checkpoint inhibitors.⁵⁹ Therefore, it is not recommended to use single-agent immunotherapy for patients with stage IV NSCLC and any activating EGFR mutation based on the latest American Society of Clinical Oncology (ASCO) and Ontario Health (OH) guidelines.¹³⁵

The exact mechanism behind the important clinical question of why EGFR-mutant NSCLC is treatment resistant to PD-1/PD-L1 inhibitors, is not yet clear, but it is considered to be associated with low tumor mutation burden (TMB), as well as an immunosuppressive TME in EGFR-signaling aberrant tumors.¹³⁶ One study retrospectively examined 171 EGFR-mutant NSCLC patients who were treated with ICIs and concluded that patients with the EGFR L858R mutation tumors had similar OS compared with patients with EGFR WT tumors, whereas patients with EGFR^{Δ19} had inferior outcomes.¹³⁷ This finding was independent of PD-L1 expression status. Another cohort of patients with sequencing data available showed that patients with the EGFR^{Δ19} mutations carried a lower TMB compared with patients with the EGFR L858R mutation. Of note, NSCLCs with high TMB are associated with a better response rate to ICIs in large scale clinical trials.^{138,139} Another study retrospectively examined 25 patients with EGFR-mutant NSCLC who received anti-PD-1 inhibitor nivolumab after disease progression on EGFR-TKI therapy, to evaluate immunotherapy efficacy and the TME. Patients with the negative EGFR T790M mutation were more likely to respond to nivolumab than those with the positive EGFR T790M mutation, possibly secondary to a higher PD-L1 expression level. However, the PFS, OS, PD-L1 expression level and tumor infiltrating CD8 T cell densities did not reach statistical significance, potentially due to the limited sample size ($n = 25$).¹⁴⁰ An evaluation of 153 patients with advanced, EGFR-mutant NSCLC (with only the EGFR^{Δ19} mutation or EGFR L858R mutation included) showed that patients with high TMB (defined as > 4.85 mutations/Mb) had shorter time-to-treatment discontinuation (TTD) and shorter OS than patients with low/intermediate TMB (low: ≤ 2.83 mutations/Mb; intermediate: 2.83–4.85 mutations/Mb).¹⁴¹ Interestingly, rebiopsied tumor tissue post TKI treatment from patients who had disease progression during EGFR-TKI treatment, showed a higher percentage of high PD-L1 expression (14% \rightarrow 28%; high expression defined as PD-L1 level $\geq 50\%$) and higher TMB (3.3 \rightarrow 4.1 mutations/Mb). Importantly, in this cohort, patients with high PD-L1 expression had a longer median PFS than patients with low PD-L1 expression after treatment of anti-PD-1 immunotherapy.¹⁴² Together, the current evidence suggests that while EGFR-mutant NSCLC responds relatively poorly to ICIs compared with EGFR WT tumors, a selected subgroup of patients with EGFR-mutant NSCLC may potentially benefit from ICIs. Further studies are

warranted to identify the group of patients with advanced EGFR-mutant lung cancers who might benefit from ICIs, such as those with high TMB or specific EGFR mutations (e.g., L858R).

Combination of EGFR-targeted therapies with ICIs

There is substantial interest to overcome the immunosuppressive effects in EGFR-mutant NSCLC, as patients who initially responded to EGFR-TKIs eventually developed treatment resistance and ICIs could potentially provide durable effects.¹⁴³ Preclinical studies demonstrated that EGFR^{Δ19} mouse tumors treated with EGFR-TKI erlotinib were infiltrated with lower frequencies of Tregs and higher frequencies of cytotoxic CD8 T cells, suggesting a more inflamed TME, compared with EGFR-mutant controls without erlotinib treatment.¹⁴⁴ Furthermore, a combination of erlotinib and anti-PD-1 therapy significantly inhibited EGFR-mutant mouse tumor growth compared with either therapy alone.¹⁴⁴ Therefore, the joint usage of EGFR-targeted therapies could potentially increase the efficacy of PD-1 blockade immunotherapy in EGFR mutant lung cancers, leading to the increasing numbers of clinical trials evaluating combinations of EGFR-targeted therapies and PD-1/PD-L1 inhibitors (Table 1).

However, several early-phase clinical trials conducted to explore the safety and efficacy of combining EGFR-TKIs and anti-PD-1/PD-L1 immunotherapies were ceased early due to severe liver or pulmonary toxicities.¹⁴⁵ Specifically, in one arm of the TATTON study (NCT02143466), combining osimertinib (a third generation EGFR TKI) and durvalumab (an anti-PD-L1 mAb) in EGFR-mutant lung cancer, 5/23 (22%) patients developed interstitial lung disease, which led to early discontinuation of the arm.¹⁴⁶ The CAURAL trial (NCT02454933), an open label, phase III trial to compare osimertinib, with or without durvalumab, was terminated early as a similar regimen used in TATTON trial caused increased incidence of interstitial pneumonitis.¹⁴⁷ In the phase I/II KEYNOTE-021 study (NCT02039674), the cohort that received pembrolizumab and gefitinib combination therapy showed high incidence (5/7 patients, 71.4%) of grade 3/4 liver toxicity.¹⁴⁵ A large database study of 20,516 participants with NSCLC in the US FDA adverse event report system reported a much higher rate of interstitial pneumonitis (18/70, 25.7%) in patients who received both EGFR-TKIs and nivolumab, compared with 4.80% for total participants and 4.59% for patients received EGFR-TKIs only.¹⁴⁸ These studies raised serious safety concerns regarding the combination of EGFR-TKIs and anti-PD-1/PD-L1 immunotherapies.

Meanwhile, several other studies reported tolerable toxicities when other combination regimens were used.^{145,149,150} In the KEYNOTE-021 study (NCT02039674), the combination of pembrolizumab and erlotinib was feasible with similar toxicities to monotherapy. The combination therapy of nivolumab and erlotinib in a phase I

trial in advanced, EGFR-mutant NSCLC patients, showed that this regimen was tolerable with durable responses in a small portion of patients (3/20, 15%) (NCT01454102). One patient, who was EGFR-TKI naïve when treated with this regimen, achieved a complete response lasting more than 5 years at the time of this report.¹⁴⁹ A phase Ib study combined the EGFR-TKI erlotinib with the anti-PD-L1 atezolizumab in 28 patients with locally advanced or metastatic NSCLC (NCT02013219).¹⁵⁰ The safety profile was tolerable, similar to erlotinib monotherapy, and the final outcome data are not yet available. In summary, these reports, although with small patient samples, suggest that certain combinations of EGFR-TKIs and anti-PD-1/PD-L1 immunotherapies are feasible and can potentially provide durable response in some patients.

Besides EGFR-TKIs, cetuximab (a chimeric mouse-human IgG1 antibody) and panitumumab (a fully humanized IgG2 antibody) are two anti-EGFR mAbs whose major mechanisms of action are blocking ligand binding, promoting receptor internalization, and mediating antibody- and complement-mediated cytotoxicity (ADCC and CDC, respectively). Cetuximab has been approved for locally advanced and recurrent and/or metastatic HNSCCs and metastatic CRCs. Cetuximab treatment upregulates PD-1 expression in NK cells, and PD-1 blockade increases cetuximab-mediated ADCC against PD-L1^{hi} HNSCC cells without EGFR amplification, indicating that the combination of anti-EGFR mAbs with ICIs could augment both innate and acquired anti-tumor immune responses against EGFR-expressing HNSCC.¹⁵¹ Currently, anti-PD-1/PD-L1 mAbs in combination with cetuximab are being evaluated in phase II clinical trials in recurrent and/or metastatic HNSCC (NCT03082534 and NCT03082534).

Ipilimumab is an anti-CTLA-4 antibody that has been approved for treating several malignancies given its role in enhancing effector cells and depleting Tregs. Few studies have explored the feasibilities and potential benefit of combining EGFR-targeted therapies and ipilimumab. In a study of patients with stage III/IV HNSCC who were treated with cetuximab, CTLA-4⁺ Tregs were found to suppress cetuximab-mediated ADCC effects and correlate with poor clinical outcome. Ipilimumab could eliminate intratumoral Tregs and restore NK cell mediated ADCC in an *ex vivo* assay, suggesting that addition of ipilimumab could improve the clinical efficacy of cetuximab treatment.¹⁵² In another study of patients with EGFR-mutant NSCLC treated with first line EGFR-TKIs, CTLA-4⁺ immune shaped cell (ISC) counts were found to be borderline correlated with OS in such patients ($P = 0.061$).⁷³ Additionally, the combination of ipilimumab plus EGFR-TKI erlotinib notably improved the PFS and OS of patients with EGFR-mutated advanced NSCLC in a phase I trial, although erlotinib plus ipilimumab caused excessive gastrointestinal toxicity resulting in early study closure.¹⁵³ Overall, more convincing evidence is warranted to support the combination therapy of EGFR-targeted therapies and anti-CTLA-4 immunotherapy. A phase 1b clinical trial is ongoing to evaluate the safety and efficacy of combining third-generation EGFR-TKI osimertinib and

Table 1 Selected clinical trials evaluating combinations of EGFR-targeted therapies and PD-1/PD-L1 inhibitors.

| Disease | Phase | treatment regimen | Outcome | Clinicaltrials.gov identifier |
|---|-------|--|---|-------------------------------|
| Advanced NSCLC | I/II | Anti-EGFR: Erlotinib (group D) ICI: Nivolumab | Unpublished | NCT02574078 |
| Stage IIIb/IV NSCLC | I | Anti-EGFR: Erlotinib (arm E) ICI: Nivolumab | ORR 15%, 24-week PFS 48% ¹⁴⁹ | NCT01454102 |
| EGFR positive, advanced NSCLC | I | Anti-EGFR: Erlotinib ICI: Atezolizumab | ORR 75%, Median PFS 15.4 months, OS 32.7 months ¹⁵⁰ | NCT02013219 |
| EGFR positive, advanced lung cancer | I | Anti-EGFR: Osimertinib ICI: Durvalumab (MEDI4736) | Terminated early due to increased incidence of interstitial lung disease ¹⁴⁶ | NCT02143466 |
| Advanced or metastatic HNSCC; NSCLC (multiple subtypes) | I/II | Anti-EGFR: CIMAvax Vaccine ^{a,158} ICI: Nivolumab or Pembrolizumab | Ongoing | NCT04298606 |
| Intermediate and high-risk locally regionally advanced HNSCC | I | Anti-EGFR: Cetuximab (arm 3) ICI: Nivolumab And radiation therapy (IMRT) | Ongoing | NCT02764593 |
| Advanced colorectal cancer | II | Anti-EGFR: Cetuximab ICI: Sintilimab And Regorafenib | Ongoing | NCT04745130 |
| Locally advanced HNSCC | I/II | Anti-EGFR: Cetuximab ICI: Durvalumab And radiation therapy (IMRT) | Ongoing | NCT03051906 |
| Recurrent/metastatic HNSCC | II | Anti-EGFR: Cetuximab ICI: Pembrolizumab | Ongoing | NCT03082534 |
| EGFR-driven advanced solid tumors | I/Ib | Anti-EGFR: BCA101 ^b ICI: Pembrolizumab | Ongoing | NCT04429542 |
| Recurrent or metastatic HNSCC (PD-L1 positive); Advanced or metastatic cutaneous SCC | I/II | Anti-EGFR: ASP-1929 ^c photoimmunotherapy ICI: Pembrolizumab or Cemiplimab | Ongoing | NCT04305795 |

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; HNSCC, head neck squamous cell carcinoma; SCC, squamous cell carcinoma.

^a CIMAvax Vaccine: CIMAvax-EGF vaccine is a therapeutic cancer vaccine, consisting of a chemical conjugate of the EGF with the P64 protein derived from the Meningitis B bacteria and Montanide ISA 51, as adjuvant.

^b BCA101: EGFR/TGF β -trap bifunctional antibody.

^c ASP-1929: antibody drug conjugate of cetuximab and IRDye 700DX[®], a phthalocyanine dye.

ipilimumab in patients with EGFR-mutant NSCLC (NCT04141644).

More preclinical and clinical studies are warranted to explore the mechanism, safety, and efficacy of EGFR-TKIs and ICIs combination therapy. With the evidence of increased incidences of toxicities in some clinical trials, especially interstitial lung diseases, exceptional precautions should be taken when designing such trials.^{143,154–156}

Conclusion and perspectives

EGFR signaling in cancer cells contributes to tumor progression by promoting cell growth, survival, and

establishing an immunosuppressive TME. Molecular-targeted therapies against EGFR currently benefit a subset of cancer patients with activating EGFR mutations. The upregulation of PD-L1 expression in tumor cells following TKI therapy might contribute to an immunosuppressive TME by inhibiting T cell-mediated antitumor cytotoxicity. Thus, EGFR-targeted therapies in combination with PD-1/PD-L1 inhibitors are being evaluated in clinical trials as a valuable therapeutic approach. To optimize PD-1/PD-L1 inhibitors in patients with aberrant EGFR signaling, it is essential to develop novel therapeutic strategies targeting the immunosuppressive pathways caused by EGFR signaling. In addition to PD-L1, the new B7/CD28 members B7-H3, B7x, and HHLA2 are also correlated with EGFR

signaling, although the underlying mechanisms remain elusive. Importantly, B7-H3,¹⁵⁷ B7x^{27,120} and HHLA2^{19,20,27} tend to have mutually exclusive expression from PD-L1 in cancer cells, suggesting that these new B7 members mediate mechanisms of tumor immune evasion that are independent from the PD-1/PD-L1 pathway. The roles of these new B7/CD28 pathways in mediating immune evasion in PD-L1-negative tumors should be explored to provide the rationale for an effective immunotherapy strategy in these tumors. Nevertheless, this may suggest that ICIs targeting these new B7 members could be an alternative option for PD-1/PD-L1 inhibitors in combination with EGFR-targeted therapies for treating patients with low or negative PD-L1 expression. Encouragingly, several inhibitors blocking these new B7 members have been under investigation in clinical trials and have shown very promising outcomes. Collectively, targeting EGFR signaling with molecular-targeted therapies in combination with cancer immunotherapies, particularly ICIs targeting new B7/CD28 members, could contribute to developing optimal cancer therapies, thus augmenting anti-tumor efficacy in the clinic.

Author contributions

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Conflict of interests

The authors declare no conflict of interest.

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