Combining immune checkpoint blockade with ErbB targeted therapies for cancer treatment

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Abstract Aberrant EGFR family signaling pathways are well known as oncogenic drivers and account for several types of cancer with their functions in abnormally promoting cell proliferation and preventing cell apoptosis. Accumulating evidence has indicated that EGFR family signaling could facilitate tumor cells to escape from immunological surveillance by reducing tumor mutation burden and inducing immunosuppressive tumor microenvironment. Therefore, ErbB driven cancers are sensitive to ErbB targeted therapies and resistant to immune checkpoint blockade (ICB). Whether combining ErbB targeted therapies and ICB can actually benefit cancer patients is still controversial. Here, we review the correlation between EGFR family signaling and cancer, summarize the recent findings in the immune related mechanisms of ErbB targeted therapies and the potential reasons why ErbB driven cancers are resistant to ICB, and further discuss the potential strategies of combining ErbB targeted therapies and ICB.
Keywords: ErbB driven cancers; ErbB targeted therapies; immune checkpoint blockade; combinational therapies
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EGFR family signaling and cancer

The ErbB receptor family belongs to the receptor tyrosine kinases (RTKs) and consists of four members: ErbB1/EGFR/HER1, ErbB2/HER2/Neu, ErbB3/HER3 and ErbB4/HER4[1]. Based on the protein structure, the ErbB receptors comprise an extracellular ligand binding domain, a hydrophobic transmembrane domain, an intracellular tyrosine kinase domain and a C-terminal tyrosine-rich tail[2]. The activity of these receptors is tightly controlled and regulated by their ligands under normal conditions. Numerous ligands of ErbB receptors have been described, including epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), amphiregulin (AREG), betacellulin (BTC), heparin binding epidermal growth factor (HB-EGF), epiregulin (EPR), neuregulins (NRGs) and epigen (EPI). EGF, TGF-α, and AREG are specific ligands only for the EGFR. BTC, HB-EGF, and EPR bind to both EGFR and ErbB4. NRG-1 and 2 selectively bind to ErbB3, and BTC, HB-EGF, EPR, NRG1-4 bind to ErbB4[3,4]. ErbB2 has no known ligand and can be activated by forming homodimers or heterodimers with other ErbB receptors[1,5,6].

Upon activation, the receptors are induced to form homo- or heterodimers, trigger the phosphorylation of intracellular domains, recruit related adaptor proteins, and subsequently activate downstream signaling[7]. The signaling pathways activated by ErbB receptors mainly comprise the PI3K-Akt-mTOR and RAS-RAF-MEK-ERK pathways, which are involved in several important physiological events including cell proliferation, apoptosis, differentiation, migration, embryonic development, and organogenesis[8]. However, abnormal activation of these pathways is considered as pro-oncogenic and the aberrations of ErbB receptors; including overexpression, amplification and active mutation, often occur in many types of cancers, such as non-small-cell lung cancer (NSCLC), metastatic colorectal cancer (mCC), glioblastoma (GBM), head and neck cancer, pancreatic, ovarian, bladder and breast cancers[9,10]. According to the prominent role of EGFR family signaling in cancer development, EGFR and its family members are very attractive therapeutic targets.

ErbB targeted therapies

Two therapeutic approaches have been employed to target ErbB receptors and inhibit their downstream signaling pathways. One is monoclonal antibodies, which are developed to directly target the extracellular domains of the receptors, block ligand binding or prevent receptor dimerization, then subsequently inhibit phosphorylation of intracellular domain and downstream signaling activation. Currently, cetuximab and panitumumab are two clinical available antibodies targeting EGFR[11]. Cetuximab is a human/mouse chimeric IgG1 antibody which has been approved for metastatic colorectal cancer (mCRC) and squamous cell carcinoma of the head and neck (SCCHN) with wild type KRAS and NRAS[12,13]. Panitumumab is a fully humanized IgG2 antibody and has been approved for the treatment of mCRC[14]. In addition, trastuzumab and pertuzumab are approved to treat ErbB2/HER2 positive tumors. Both of these two are humanized antibodies, trastuzumab targets the extracellular domain of ErbB2 to induce the internalization of cell surface ErbB2[15], while pertuzumab targets the dimerization arm of extracellular ErbB2 domain to block its dimerization with other ErbB receptors[16].

The second therapeutic approach is tyrosine kinase inhibitors (TKIs), which are designed to bind the intracellular tyrosine kinase domain (TKD) of the receptors; thereby, blocking the activation of downstream signaling pathways[9,17]. The TKIs of ErbB receptor have been developed to the third generation. The first generation, gefitinib, erlotinib and lapatinib, can target the receptor through competitive and reversible binding at the tyrosine kinase domain[18]. Unlike the first generation, the second generation TKIs, afatinib, dacomitinib and neratinib, have the advantage of forming covalent, irreversible bonds with the target domain, and this will increase their effectiveness through a prolonged inhibition of ErbB signaling[19-21]. The most common mechanism of resistance to first- or second-generation TKIs is the development of a mutation in the EGFR gene called T790M. Therefore, the third generation TKIs, such as osimertinib and olmutinib, are designed to target this specific mutation[22,23]. Lapatinib is currently clinically approved for patients with ErbB2/HER2 positive breast cancer[24]. Gefitinib, erlotinib, afatinib, and osimertinib are currently clinically approved for the treatment of EGFR driven NSCLC[7], and erlotinib is also approved for use in pancreatic cancer in combination with gemcitabine[25,26]. Clinical studies have shown that ErbB-targeted therapies, especially new generation of irreversible EGFR TKIs, could significantly improve the outcome of patients with late-stage ErbB-dependent cancers and had a high response rate. However, almost all patients eventually relapse and develop resistance to treatment; no reports on a cure have yet been seen[27]. A major challenge for EGFR TKI treatment is developing new inhibitors or combinational therapies that can overcome drug resistance and limit tumor relapses.

In addition to inhibiting tumor growth by inducing tumor cell apoptosis through directly blocking oncogenic signaling, recent studies indicate that the antitumor efficacy of these ErbB targeted therapeutics also relies on the host immune system. Trastuzumab and cetuximab have been reported to mediate tumor killing by engaging several innate immune effectors, including complement-dependent cytotoxicity (CDC), antibody-dependent cell cytotoxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP) by myeloid cells, and NK cells[28]. Moreover, both clinical and preclinical studies have observed that the antitumor efficacy of ErbR2/HER2 and EGFR targeted antibodies strongly relay on the host adaptive T cell immunity[29-32]. EGFR TKIs have also been reported to influence the host immune system, either by modulating tumor plasticity to enhance immune cell attack[33], or altering tumor microenvironment, including reducing regulatory T cells[34] and increasing $CD8^+$ T cells infiltration[35]. Whereas another study reported that EGFR TKIs could inhibit T cell activation through inhibiting the c-Raf/ERK (extracellular signal–regulated kinase) and AKT signaling

pathways *in vitro*[36]. The mechanisms by which EGFR TKIs influence the host immune system are still not well defined. Interestingly, our recent study observed that manipulating the dosing and timing of EGFR TKIs could enhance the antitumor efficacy in host T cell immunity dependent fashion. We demonstrated that compared with standard of care hyperfractionated EGFR TKI treatment (low dose with daily treatment, HyperTKI), hypofractionated EGFR TKI treatment (high dose with a low frequency treatment, HypoTKI) not only could significantly reduce tumor burden, but also could enhance tumor specific T cell responses through triggering great innate sensing for type I IFN and CXCL10 production[37]. Further investigations with syngeneic mouse models need to be done to explore whether EGFR TKIs have other impacts on the host immune system.

Immune checkpoint blockade therapy for ErbB related cancers

Accumulating clinical evidence has proven that cancer immunotherapy is a promising treatment for patients with advanced cancers. Due to the great success at the clinical level, cancer immunotherapy was named as "Breakthrough of the Year" by Science in 2013[38,39].Of even greater concern, the Nobel prize for physiology or medicine in 2018 was awarded to James P. Allison and Tasuku Honjo for their discovery of cytotoxic T-lymphocyte-associated protein (CTLA-4) and programmed cell death protein 1 / programmed cell death protein ligand 1 (PD-1 / PD-L1), the inhibitory antibodies of which have been widely and successfully used in clinic as cancer immunotherapy and have been approved for the treatment of at least ten types of cancers, such as melanoma, lung cancer, CRC, head and neck squamous cell carcinoma (HNSCC), Hodgkin's lymphoma, urothelial carcinoma, gastric cancer, and cervical cancer[23,40,41]. Immunotherapy, especially PD-L1/PD-1 immune checkpoint blockade (ICB), has shown encouraging antitumor efficacy in lung cancer patients, including both NSCLC and SCLC. However, several clinical trials have shown that PD-L1/PD-1 blockade did not benefit cancer patients with EGFR driver mutation regardless of PD-L1 expression[42,43]. The underlining mechanisms are still largely unclear. PD-L1 expression level, tumor mutation burden (TMB) and immunosuppressive tumor microenvironment (TME) are the most potential reasons.

Previous preclinical study has reported that EGFR active mutation could induce PD-L1 expression in lung epithelial cells, and EGFR TKI could reverse this effect[44]. Later, some studies confirmed this correlation with using the tumor samples from clinical patients[45-47]. In contrast, there are also some studies demonstrated that PD-L1 expression in tumor tissues was negatively correlated with EGFR mutation status. A pooled analysis found that compared to NSCLC patients with wild type EGFR, patients with EGFR mutations were less likely to be PD-L1-positive[48]. In addition, the analysis of the Cancer Genome Atlas and the Guangdong Lung Cancer Institute (GCLI) database have also confirmed the lower PD-L1 expression in tumors with EGFR mutation[49,50]. Therefore, the relationship between EGFR mutation and PD-L1 expression is still conflicting, PD-L1 expression may not be the major reason why NSCLC cancer patients with EGFR mutation show poor response to PD-1/PD-L1 blockade.

Tumor mutation burden (TMB) is becoming a promising biomarker for the responses to immunotherapy in cancer patients[51-54]. Low TMB is correlated with low response to immunotherapy, which suggests that EGFR mutant NSCLC tumor may harbor low TMB. Indeed, studies have reported that patients with *EGFR* mutations showed a significantly decreased TMB [49]. In addition, the EGFR mutation status is associated with TMB, NSCLC tumors with $EGFR^{\Delta 19}$alterations harbored lower TMB compared with $EGFR^{L858R}$ tumors[43,55]. Consistently, NSCLC patients with $EGFR^{L858R}$ have better response to PD-L1/PD-1 blockade than patients with $EGFR^{\Delta 19}$alterations[43]. Therefore,

low TMB is one of the reasons for the low response rate to immunotherapy.

Tumor microenvironment (TME) is another key factor that impacts the patients' response rate to immunotherapy[56]. Based on the presence/absence and the location of tumor infiltrating lymphocytes (TIL), tumors can be classified into three types: immune-desert TME, immune-excluded TME, and the inflamed TME. Immune-desert and immune-excluded TME are also considered to be uninflamed TME, which has been reported to be resistant to ICB, whereas tumors with inflamed TME are sensitive to ICB[57]. Indeed, it has been reported that EGFR active mutations were associated with the uninflamed TME[49], and EGFR signaling pathway activation in immunosuppressive cells was related to the immunosuppressive TME[50]. Previous studies have revealed that EGFR signaling activation played vital roles in generating regulatory T cells (Tregs) and maintaining their suppressive functions[58,59]. In addition, other studies also showed that STAT3 (downstream of EGFR) activation could inhibit the maturation of dendritic cells (DCs), and promote the IDO expression in both DCs and myeloid-derived suppressor cells (MDSCs)[60-62]. However, how the activated EGFR signaling can be transferred from tumor cells to immunosuppressive cells is still not well studied. A recent study has observed that EGFR expressed on tumor cells could be transferred to Tregs through exosomes and then promote the generation of tumor specific Tregs[63]. This also indicated that exosomes could transfer EGFR to DCs and MDSCs for their immunosuppressive function. Taken together, all these studies suggest that tumor cells with activated EGFR signaling can induce the immunosuppressive TME to mediate immune tolerance for tumor development and resistance to immunotherapy.

Combination of EGFR targeted therapies and immune checkpoint blockade

Reducing tumor burden and increasing immunogenicity are considered as two key factors for effective tumor control[64]. Combinational strategies that achieve these two goals will improve the outcomes of patients clinically[65]. EGFR targeted therapies, especially EGFR TKIs, have shown promising antitumor efficacy in cancer patients with EGFR active mutations. However, the major problem for EGFR targeted therapies is that they can initially reduce tumor burden and significantly improve the outcomes, but patients eventually encounter tumor relapse and develop drug resistance to EGFR TKIs[27]. While the strength of emerging immunotherapies, especially ICB, is the long-term response during the treatment, the weakness is low response rate and only a small fraction of patients respond to treatment[65]. According to the strength and weakness of EGFR targeted therapies and ICB, combination of these two therapies may achieve both initial high response rate and later long-term response to treatment, improved initial outcomes and overall survival of cancer patients[37]. Notably, many clinical trials with the combination of EGFR TKIs and ICB have been conducted. Although combinational treatment showed promising synergistic antitumor effect, several trials have been terminated due to severe toxicities[66]. One study showed that combination of Eroltinib with ICB could benefit patients with tolerable toxicity[67]. Another study showed that sequential PD-L1/PD-1 blockade and Osimertinib could cause severe immune related side effects[68]. Interestingly, our recent study proposed that optimizing the dosing and timing of EGFR TKI and ICB could achieve both efficient tumor control without severe toxicities. We demonstrated that combining HypoTKI and PD-L1 blockade could effectively control advanced large tumors, increase overall survival, and limit tumor relapse. We also observed that compared with HyperTKI plus PD-L1 blockade, HypoTKI plus PD-L1 blockade has fewer side effects[37]. Therefore, several factors, such as selected EGFR TKIs, manipulating the dosing and timing of EGFR TKI, and the schedule of treatment, need to be considered to optimize the combinational strategies of EGFR targeted therapies and ICB to achieve the maximized antitumor effects without severe side effects.

Conclusion remarks

ErbB driven cancer patients are resistant to emerging ICB treatment. ErbB targeted therapies, especially EGFR TKIs, are still considered the frontline therapies. According to the strength of EGFR TKI in reducing tumor burden and the long-lasting response of ICB, combination of EGFR TKIs and ICB has been tested in clinical trials; however, severe toxicities resulted in the premature termination of several trials. Although a preclinical study has shown that optimizing the dosing and timing of EGFR TKI and ICB could achieve synergistic antitumor effect without severe side effects in mouse models, whether the proposed strategy can be applied to the clinic still needs further investigations. Moreover, to explore the reasons of toxicities caused by the EGFR TKI and ICB will facilitate the development of potential combination strategies for the clinic.

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Competing interests

The authors declare that they have no competing interests.

Reference

1 Hynes, N. E. & MacDonald, G. ErbB receptors and signaling pathways in cancer. *Curr Opin Cell Biol* **21**, 177-184, doi:10.1016/j.ceb.2008.12.010 (2009).

2 Wee, P. & Wang, Z. Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways. *Cancers (Basel)* **9**, doi:10.3390/cancers9050052 (2017).

3 Normanno, N. *et al.* Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* **366**, 2-16, doi:10.1016/j.gene.2005.10.018 (2006).

4 Singh, B., Carpenter, G. & Coffey, R. J. EGF receptor ligands: recent advances. *F1000Res* **5**, doi:10.12688/f1000research.9025.1 (2016).

5 Ou, S. I. *et al.* HER2 Transmembrane Domain (TMD) Mutations (V659/G660) That Stabilize Homo- and Heterodimerization Are Rare Oncogenic Drivers in Lung Adenocarcinoma That Respond to Afatinib. *J Thorac Oncol* **12**, 446-457, doi:10.1016/j.jtho.2016.11.2224 (2017).

6 Yarden, Y. & Sliwkowski, M. X. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* **2**, 127-137, doi:10.1038/35052073 (2001).

7 Jacobi, N., Seeboeck, R., Hofmann, E. & Eger, A. ErbB Family Signalling: A Paradigm for Oncogene Addiction and Personalized Oncology. *Cancers (Basel)* **9**, doi:10.3390/cancers9040033 (2017).

8 Roskoski, R., Jr. The ErbB/HER family of protein-tyrosine kinases and cancer. *Pharmacol Res* **79**, 34-74, doi:10.1016/j.phrs.2013.11.002 (2014).

9 Holbro, T., Civenni, G. & Hynes, N. E. The ErbB receptors and their role in cancer progression. *Exp Cell Res* **284**, 99-110, doi:10.1016/s0014-4827(02)00099-x (2003).

10 Appert-Collin, A., Hubert, P., Cremel, G. & Bennasroune, A. Role of ErbB Receptors in Cancer Cell Migration and Invasion. *Front Pharmacol* **6**, 283, doi:10.3389/fphar.2015.00283 (2015).

11 Ohhara, Y. *et al.* Role of targeted therapy in metastatic colorectal cancer. *World J Gastrointest Oncol* **8**, 642-655, doi:10.4251/wjgo.v8.i9.642 (2016).

12 Lievre, A. *et al.* KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* **66**, 3992-3995, doi:10.1158/0008-5472.CAN-06-0191 (2006).

13 Kim, E. S., Khuri, F. R. & Herbst, R. S. Epidermal growth factor receptor biology (IMC-C225). *Curr Opin Oncol* **13**, 506-513, doi:10.1097/00001622-200111000-00014 (2001).

14 Cohenuram, M. & Saif, M. W. Panitumumab the first fully human monoclonal antibody: from the bench to the clinic. *Anticancer Drugs* **18**, 7-15, doi:10.1097/CAD.0b013e32800feecb (2007).

15 Cuello, M. *et al.* Down-regulation of the erbB-2 receptor by trastuzumab (herceptin) enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in breast and ovarian cancer cell lines that overexpress erbB-2. *Cancer Res* **61**, 4892-4900 (2001).

16 Franklin, M. C. *et al.* Insights into ErbB signaling from the structure of the ErbB2-pertuzumab complex. *Cancer Cell* **5**, 317-328, doi:10.1016/s1535-6108(04)00083-2 (2004).

17 Yamaoka, T., Ohba, M. & Ohmori, T. Molecular-Targeted Therapies for Epidermal Growth Factor Receptor and Its Resistance Mechanisms. *Int J Mol Sci* **18**, doi:10.3390/ijms18112420 (2017).

18 Modjtahedi, H. & Essapen, S. Epidermal growth factor receptor inhibitors in cancer treatment: advances, challenges and opportunities. *Anticancer Drugs* **20**, 851-855, doi:10.1097/CAD.0b013e3283330590 (2009).

19 Sequist, L. V. *et al.* Neratinib, an irreversible pan-ErbB receptor tyrosine kinase inhibitor: results of a phase II trial in patients with advanced non-small-cell lung cancer. *J Clin Oncol* **28**, 3076-3083, doi:10.1200/JCO.2009.27.9414 (2010).

20 Engelman, J. A. *et al.* PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. *Cancer Res* **67**, 11924-11932, doi:10.1158/0008-5472.CAN-07-1885 (2007).

21 Miller, V. A. *et al.* Afatinib versus placebo for patients with advanced, metastatic non-small-cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-Lung 1): a phase 2b/3 randomised trial. *Lancet Oncol* **13**, 528-538, doi:10.1016/S1470-2045(12)70087-6 (2012).

22 Lamb, Y. N. & Scott, L. J. Osimertinib: A Review in T790M-Positive Advanced Non-Small Cell Lung Cancer. *Target Oncol* **12**, 555-562, doi:10.1007/s11523-017-0519-0 (2017).

23 Seebacher, N. A., Stacy, A. E., Porter, G. M. & Merlot, A. M. Clinical development of targeted and immune based anti-cancer therapies. *J Exp Clin Cancer Res* **38**, 156, doi:10.1186/s13046-019-1094-2 (2019).

24 Johnston, S. R. & Leary, A. Lapatinib: a novel EGFR/HER2 tyrosine kinase inhibitor for cancer. *Drugs Today (Barc)* **42**, 441-453, doi:10.1358/dot.2006.42.7.985637 (2006).

25 Sinn, M. *et al.* CONKO-005: Adjuvant Chemotherapy With Gemcitabine Plus Erlotinib Versus Gemcitabine Alone in Patients After R0 Resection of Pancreatic Cancer: A Multicenter Randomized Phase III Trial. *J Clin Oncol* **35**, 3330-3337, doi:10.1200/JCO.2017.72.6463 (2017).

26 Shin, S., Park, C. M., Kwon, H. & Lee, K. H. Erlotinib plus gemcitabine versus gemcitabine for pancreatic cancer: real-world analysis of Korean national database. *BMC Cancer* **16**, 443, doi:10.1186/s12885-016-2482-z (2016).

27 Wu, S. G. & Shih, J. Y. Management of acquired resistance to EGFR TKI-targeted therapy in advanced non-small cell lung cancer. *Mol Cancer* **17**, 38, doi:10.1186/s12943-018-0777-1 (2018).

28 Michelle Xu, M., Pu, Y., Weichselbaum, R. R. & Fu, Y. X. Integrating conventional and antibody-based targeted anticancer

treatment into immunotherapy. *Oncogene* **36**, 585-592, doi:10.1038/onc.2016.231 (2017).

29 Arnould, L. *et al.* Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? *Br J Cancer* **94**, 259-267, doi:10.1038/sj.bjc.6602930 (2006).

30 Park, S. *et al.* The Therapeutic Effect of Anti-HER2/neu Antibody Depends on Both Innate and Adaptive Immunity. *Cancer Cell* **18**, 160-170, doi:10.1016/j.ccr.2010.06.014 (2010).

31 Correale, P. *et al.* Cetuximab +/- chemotherapy enhances dendritic cell-mediated phagocytosis of colon cancer cells and ignites a highly efficient colon cancer antigen-specific cytotoxic T-cell response in vitro. *International Journal of Cancer* **130**, 1577-1589, doi:10.1002/ijc.26181 (2012).

32 Pozzi, C. *et al.* The EGFR-specific antibody cetuximab combined with chemotherapy triggers immunogenic cell death. *Nat Med* **22**, 624-631, doi:10.1038/nm.4078 (2016).

33 Dominguez, C., Tsang, K. Y. & Palena, C. Short-term EGFR blockade enhances immune-mediated cytotoxicity of EGFR mutant lung cancer cells: rationale for combination therapies. *Cell Death Dis* **7**, e2380, doi:10.1038/cddis.2016.297 (2016).

34 Mascia, F. *et al.* Cell autonomous or systemic EGFR blockade alters the immune-environment in squamous cell carcinomas. *Int J Cancer* **139**, 2593-2597, doi:10.1002/ijc.30376 (2016).

35 Thress, K. S. *et al.* Modulation of Biomarker Expression by Osimertinib: Results of the Paired Tumor Biopsy Cohorts of the AURA Phase I Trial. *J Thorac Oncol* **12**, 1588-1594, doi:10.1016/j.jtho.2017.07.011 (2017).

36 Luo, Q. *et al.* Erlotinib inhibits T-cell-mediated immune response via down-regulation of the c-Raf/ERK cascade and Akt signaling pathway. *Toxicol Appl Pharmacol* **251**, 130-136, doi:10.1016/j.taap.2010.12.011 (2011).

37 Liu, Z. *et al.* Hypofractionated EGFR tyrosine kinase inhibitor limits tumor relapse through triggering innate and adaptive immunity. *Sci Immunol* **4**, doi:10.1126/sciimmunol.aav6473 (2019).

38 Couzin-Frankel, J. Breakthrough of the year 2013. Cancer immunotherapy. *Science* **342**, 1432-1433, doi:10.1126/science.342.6165.1432 (2013).

39 Sambi, M., Bagheri, L. & Szewczuk, M. R. Current Challenges in Cancer Immunotherapy: Multimodal Approaches to Improve Efficacy and Patient Response Rates. *J Oncol* **2019**, 4508794, doi:10.1155/2019/4508794 (2019).

40 Altmann, D. M. A Nobel Prize-worthy pursuit: cancer immunology and harnessing immunity to tumour neoantigens. *Immunology* **155**, 283-284, doi:10.1111/imm.13008 (2018).

41 Santaniello, A. *et al.* Tumour Microenvironment and Immune Evasion in EGFR Addicted NSCLC: Hurdles and Possibilities. *Cancers (Basel)* **11**, doi:10.3390/cancers11101419 (2019).

42 Fruh, M. & Peters, S. EGFR mutation subtype impacts efficacy of immune checkpoint inhibitors in Non-Small Cell Lung Cancer. *Ann Oncol*, doi:10.1093/annonc/mdz185 (2019).

43 Hastings, K. *et al.* EGFR mutation subtypes and response to immune checkpoint blockade treatment in non-small cell lung cancer. *Ann Oncol*, doi:10.1093/annonc/mdz141 (2019).

44 Akbay, E. A. *et al.* Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. *Cancer Discov* **3**, 1355-1363, doi:10.1158/2159-8290.CD-13-0310 (2013).

45 Tang, Y. *et al.* The association between PD-L1 and EGFR status and the

prognostic value of PD-L1 in advanced non-small cell lung cancer patients treated with EGFR-TKIs. *Oncotarget* **6**, 14209-14219, doi:10.18632/oncotarget.3694 (2015).

46 Azuma, K. *et al.* Association of PD-L1 overexpression with activating EGFR mutations in surgically resected nonsmall-cell lung cancer. *Ann Oncol* **25**, 1935-1940, doi:10.1093/annonc/mdu242 (2014).

47 D'Incecco, A. *et al.* PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. *Br J Cancer* **112**, 95-102, doi:10.1038/bjc.2014.555 (2015).

48 Soo, R. A. *et al.* Immune checkpoint inhibitors in epidermal growth factor receptor mutant non-small cell lung cancer: Current controversies and future directions. *Lung Cancer* **115**, 12-20, doi:10.1016/j.lungcan.2017.11.009 (2018).

49 Dong, Z. Y. *et al.* EGFR mutation correlates with uninflamed phenotype and weak immunogenicity, causing impaired response to PD-1 blockade in non-small cell lung cancer. *Oncoimmunology* **6**, e1356145, doi:10.1080/2162402X.2017.1356145 (2017).

50 Yu, S., Liu, D., Shen, B., Shi, M. & Feng, J. Immunotherapy strategy of EGFR mutant lung cancer. *Am J Cancer Res* **8**, 2106-2115 (2018).

51 Chan, T. A. *et al.* Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Ann Oncol* **30**, 44-56, doi:10.1093/annonc/mdy495 (2019).

52 Fancello, L., Gandini, S., Pelicci, P. G. & Mazzarella, L. Tumor mutational burden quantification from targeted gene panels: major advancements and challenges. *J Immunother Cancer* **7**, 183, doi:10.1186/s40425-019-0647-4 (2019).

53 Wu, Y. *et al.* The Predictive Value of Tumor Mutation Burden on Efficacy of Immune Checkpoint Inhibitors in Cancers: A Systematic Review and Meta-Analysis. *Front Oncol* **9**, 1161, doi:10.3389/fonc.2019.01161 (2019).

54 Cao, D., Xu, H., Xu, X., Guo, T. & Ge, W. High tumor mutation burden predicts better efficacy of immunotherapy: a pooled analysis of 103078 cancer patients. *Oncoimmunology* **8**, e1629258, doi:10.1080/2162402X.2019.1629258 (2019).

55 Offin, M. *et al.* Tumor Mutation Burden and Efficacy of EGFR-Tyrosine Kinase Inhibitors in Patients with EGFR-Mutant Lung Cancers. *Clin Cancer Res* **25**, 1063-1069, doi:10.1158/1078-0432.CCR-18-1102 (2019).

56 Teng, M. W., Ngiow, S. F., Ribas, A. & Smyth, M. J. Classifying Cancers Based on T-cell Infiltration and PD-L1. *Cancer Res* **75**, 2139-2145, doi:10.1158/0008-5472.CAN-15-0255 (2015).

57 Chen, D. S. & Mellman, I. Elements of cancer immunity and the cancer-immune set point. *Nature* **541**, 321-330, doi:10.1038/nature21349 (2017).

58 Wang, S. *et al.* Amphiregulin Confers Regulatory T Cell Suppressive Function and Tumor Invasion via the EGFR/GSK-3beta/Foxp3 Axis. *J Biol Chem* **291**, 21085-21095, doi:10.1074/jbc.M116.717892 (2016).

59 Zaiss, D. M. *et al.* Amphiregulin enhances regulatory T cell-suppressive function via the epidermal growth factor receptor. *Immunity* **38**, 275-284, doi:10.1016/j.immuni.2012.09.023 (2013).

60 Ogasawara, N. *et al.* Hemoglobin induces the expression of indoleamine 2,3-dioxygenase in dendritic cells through the activation of PI3K, PKC, and NF-kappaB and the generation of reactive oxygen species. *J Cell Biochem* **108**, 716-725, doi:10.1002/jcb.22308 (2009).

61 Yu, J. *et al.* Myeloid-derived suppressor cells suppress antitumor immune

responses through IDO expression and correlate with lymph node metastasis in patients with breast cancer. *J Immunol* **190**, 3783-3797, doi:10.4049/jimmunol.1201449 (2013).

62 Park, S. J. *et al.* IL-6 regulates in vivo dendritic cell differentiation through STAT3 activation. *J Immunol* **173**, 3844-3854, doi:10.4049/jimmunol.173.6.3844 (2004).

63 Huang, S. H., Li, Y., Zhang, J., Rong, J. & Ye, S. Epidermal growth factor receptor-containing exosomes induce tumor-specific regulatory T cells. *Cancer Invest* **31**, 330-335, doi:10.3109/07357907.2013.789905 (2013).

64 Zappasodi, R., Merghoub, T. & Wolchok, J. D. Emerging Concepts for Immune Checkpoint Blockade-Based Combination Therapies. *Cancer Cell* **33**, 581-598, doi:10.1016/j.ccell.2018.03.005 (2018).

65 Wargo, J. A., Cooper, Z. A. & Flaherty, K. T. Universes collide: combining immunotherapy with targeted therapy for cancer. *Cancer Discov* **4**, 1377-1386, doi:10.1158/2159-8290.CD-14-0477 (2014).

66 Ahn, M. J., Sun, J. M., Lee, S. H., Ahn, J. S. & Park, K. EGFR TKI combination with immunotherapy in non-small cell lung cancer. *Expert Opin Drug Saf* **16**, 465-469, doi:10.1080/14740338.2017.1300656 (2017).

67 Yang, J. C. *et al.* Pembrolizumab in Combination With Erlotinib or Gefitinib as First-Line Therapy for Advanced NSCLC With Sensitizing EGFR Mutation. *J Thorac Oncol* **14**, 553-559, doi:10.1016/j.jtho.2018.11.028 (2019).

68 Schoenfeld, A. J. *et al.* Severe immune-related adverse events are common with sequential PD-(L)1 blockade and osimertinib. *Ann Oncol* **30**, 839-844, doi:10.1093/annonc/mdz077 (2019).