STAT3 as a potential immunotherapy biomarker in oncogene-addicted non-small cell lung cancer

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Abstract: Immune checkpoint blockade has modified the treatment landscape for many types of tumors, including lung cancer. Still our knowledge on the biology of the interaction between tumor cells and the microenvironment is limited, preventing the optimal use of these new compounds and the maximum benefit that the patients can derive from them. We have actively worked on the role of STAT3, a transcriptional factor that causes innate resistance to targeted therapies in oncogene-addicted tumors. In this short review we take the opportunity to express our opinion and review existing knowledge on the immune role of STAT3 and the possible implications that this may have for the discovery of new biomarkers to predict response to immunotherapy, as well as new partners to combine with and increase the efficacy of immune checkpoint inhibitors.

Keywords: biomarkers, immunotherapy, lung cancer, PD-L1, STAT3

Introduction

Immune checkpoint inhibitors, like anti-programmed cell death-1 (PD-1) or anti-programmed death-ligand 1 (PD-L1) antibodies, have demonstrated positive results in non-small cell lung cancer (NSCLC) patients, with prolonged overall survival and durable responses. Nevertheless, the response rate to immunotherapy does not exceed 30% in the second-line setting, or 45% in the first-line setting. Tumor recurrences are common clinical findings, and disease progression can occur even after a durable response, but there is little knowledge about the complex mechanisms of resistance to immune checkpoint inhibitors. Surprisingly, the T-cell activation through immune checkpoint blockade may trigger the proliferation of cancer cells in certain tumor types that are dependent on T cells. Therefore, greater effort should be made in order to raise the bar of efficacy and identify patients who can substantially benefit from therapies targeting the immune system. Combination strategies appear to be an interesting approach to optimize immunotherapy treatment outcome in NSCLC patients.

An example is the combination of chemotherapy with the anti-PD-1 antibody, pembrolizumab, that has been approved as first-line treatment in patients with lung adenocarcinoma. Many clinical trials combining anti-PD-1/PD-L1 antibodies with other immune checkpoint inhibitors are also ongoing. The discovery of predictive biomarkers is necessary for improving the efficacy of immunotherapy. Until now, great attention has been paid to the expression of PD-L1 as a predictive marker of response to immune checkpoint blockade. Immunohistochemistry evaluation of PD-L1 expression is the only available biomarker in the clinical setting, but its role is still controversial. High tumor mutational burden is also related to the outcome of immune checkpoint blockade.

With the exception of KRAS and BRAF-mutant NSCLC, other lung adenocarcinomas driven by oncogenic alterations, like epidermal growth factor receptor (EGFR) mutations or echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) fusions, have
low mutational burden\textsuperscript{16} and seem to derive minimal benefit from immunotherapy.\textsuperscript{17}

We and others have highlighted the role of signal transducer and activator of transcription 3 (STAT3) and yes-associated protein 1 (YAP1)-Src in innate resistance to \textit{EGFR} tyrosine kinase inhibitors (TKIs) in \textit{EGFR}-mutant NSCLC.\textsuperscript{18–22} We are currently defining the regulatory role of STAT3 and YAP1-Src on the expression and activation of receptor tyrosine kinases (RTKs) in \textit{EGFR} mutants,\textsuperscript{23} as well as other oncogene-addicted tumors.\textsuperscript{24} In this short review we will summarize the available data on STAT3 and PD-L1 and provide a rationale for STAT3 as a potential biomarker as well as a target for optimizing treatment with immune checkpoint inhibitors.

\textbf{STAT3 signaling and antitumor immunity}  
It is well recognized that STAT3 plays a critical role in cancer.\textsuperscript{25–27} It also affects many aspects of the immune system.\textsuperscript{28} STAT3 induces PD-L1 up-regulation in many tumors and therefore assists cancer cells to escape immune surveillance.\textsuperscript{29,30} It binds directly to the promoter of PD-L1, both in antigen-presenting cells\textsuperscript{31} and in tumor cells.\textsuperscript{32} It also activates DNA methyltransferase 1 (DNMT1), which methylates the promoter region, and subsequently suppresses the expression of immunoproteasome (PSM) subunits and major histocompatibility complex (MHC) molecules. In contrast, STAT1, which is negatively regulated by STAT3, induces the expression of PSM subunits and MHC molecules\textsuperscript{33} (Figure 1). PSMs generate peptides on the cell surface that fit in the groove of MHC molecules and allow surveillance by CD8 T cells.\textsuperscript{34} PSMB8 and PSMB9 expression is higher in \textit{EGFR}-mutant than in \textit{EGFR}-wildtype NSCLC cell lines, suggesting a stronger role of STAT3 in immune suppression in \textit{EGFR}-mutant lung cancer.\textsuperscript{33}

STAT3 is phosphorylated at tyrosine 705 position through Janus kinase 1/2 (JAK1/2). This phosphorylation mediates the formation of STAT3-STAT3 homodimers, which translocate to the nucleus and bind to DNA promoting oncogenic functions in tumor cells.\textsuperscript{35,36} In the nucleus, the phosphorylation at serine 727 is mediated by TC45 (protein tyrosine phosphatase nonreceptor type 2, PTPN2) and inactivates pY705.\textsuperscript{37} Conversely, the activity of STAT3 in the immune cells is also regulated by dual specificity phosphatase 2 (DUSP2).\textsuperscript{38} It seems that naïve CD4-positive T cells undergo T-helper 17 (Th17) differentiation independently of JAK activity.\textsuperscript{39} Moreover, Th17 are negatively regulated by DUSP2 activation and subsequent STAT3 dephosphorylation,\textsuperscript{38} suggesting a role of STAT3 activation in promoting Th17 differentiation which is dependent on DUSP2 rather than on JAK. Surprisingly, but in line with the concept of alternative mechanisms of action in immune or nonimmune tumor cells, the proliferation signal is driven by p-S727, and not p-Y705, in chronic lymphocytic leukemia cells.\textsuperscript{40}

Thus, STAT3 has been implicated in regulating the tumor microenvironment through several mechanisms, including the recruitment of myeloid-derived suppressor cells (MDSCs) or the decrease of immune cell infiltration, in different types of tumors.\textsuperscript{41–46} Tumor-derived factors can activate JAK/STAT3 signaling in hematopoietic cells, leading to abnormal differentiation of dendritic cells, which are thus unable to activate CD8 T cells. On the other hand, abnormal dendritic cells can promote T-cell tolerance and immunosuppressive immature myeloid cells.\textsuperscript{47–49} Of note, they show increased PD-L1 expression.\textsuperscript{49} This mechanism of STAT3 mediated dysfunction of dendritic cells was also found in a NSCLC model.\textsuperscript{50}

\textbf{The role of interferon \gamma}  
Interferon \gamma (IFN\textgreek{g}), encoded by the \textit{IFNG} gene, is secreted by macrophages and other immune infiltrating cells\textsuperscript{51} and promotes PD-L1 expression.\textsuperscript{10} We have reported that high baseline IFN\textgreek{g} mRNA levels can be related to better outcome of NSCLC and melanoma patients treated with the anti-PD-1 antibodies, nivolumab or pembrolizumab, respectively.\textsuperscript{52} IFN\textgreek{g} binds to its receptor and activates STAT1 through JAK1/2, but it can also activate STAT3 with opposite biological effect.\textsuperscript{51,53} IFN\textgreek{g}-mediated STAT3 activation requires Src activity and is weaker in wildtype STAT1 expressing cells than in STAT1-null.\textsuperscript{53} The relative abundance of these two members of STAT family in tumor cells can justify the different IFN\textgreek{g}-mediated activation patterns. IFNy requires cyclin-dependent kinase 5 (CDK5) and its activator, p35, to induce the expression of PD-L1.\textsuperscript{54,55} When CDK5 is lost, the IFN regulatory factors IRF2 and IRF2 binding protein 2 (IRF2BP2) persist and negatively regulate PD-L1 expression\textsuperscript{54,55} (Figure 1). Activated STAT1
Programmed death-ligand 1 (PD-L1) is the ligand of programmed death 1 (PD-1) receptor. PD-1 is expressed on the T-cell surface [mainly CD8+ T-lymphocytes], while PD-L1 is presented on the cell surface by antigen-presenting cells (APCs; as macrophages) and tumor cells. Epidermal growth factor receptor (EGFR)-activating mutations are located in the tyrosine kinase domains and mainly in the form of a base-pair deletion at exon 19 (ΔE746_A750) or a point mutation at exon 21 (L858R). Anaplastic lymphoma kinase (ALK) rearrangements involve gene fusion partners, leading to constitutive protein activation. In NSCLC the echinoderm microtubule-associated protein-like 4 (EML4)-ALK variant is the most frequently reported fusion gene. The MET receptor can be hyperactivated through gene amplification (copy number gain), or gene splicing variants. EGFR, ALK and MET signal via tyrosine phosphorylation lead to the activation of mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription 3 (STAT3) and AKT pathways. MAPK pathway is mainly dependent on Src homology 2 domain-containing phosphotyrosine phosphatase 2 (SHP2). SHP2 interferes with the process of Ras inactivation catalyzed by Ras GTPase-activating protein (RasGAP), hence increasing the half-life of activated Ras (GTP-Ras). MAPK/ERK Kinase 1 (MEK1) and MEK2 are activated upon phosphorylation by Ras cascade. MEK1/2 is in turn able to phosphorylate and activate extracellular signal-regulated kinase (ERK1 and ERK2). AKT is the major downstream target of phosphatidylinositol 3-kinase (PI3K), which is activated by the receptor tyrosine kinases (RTKs). Activated PI3K induces the recruitment of AKT to the cell membrane, driving a conformational change in the protein. This enables full activation of AKT upon phosphorylation. Following activation, AKT translocates to the cytoplasm and nucleus, and phosphorylates various downstream substrates including mammalian target of rapamycin (mTOR). Both ERK and AKT can induce up-regulation of PD-L1. The specific mechanisms of this activation pathway are still not clearly defined. SHP2 activates several Src family kinase (SFKs), including Src itself. Upon Src activation, several downstream Src binding partners are targeted for phosphorylation, including yes-associated protein 1 (YAP1). YAP1 is a transcriptional coactivator, and has been reported to bind several DNA-binding transcription factors, thus mediating up-regulation of several RTKs. Signaling transducer and activator of transcription 3 (STAT3) can be activated through Janus-like kinase (JAK) only in the presence of Src kinase activity, in low STAT1 conditions. When activated, STAT3 undergoes phosphorylation-induced homodimerization. The homodimer translocates to the nucleus and binds to DNA. Through the activation of DNA methyltransferase 1 (DNMT1), STAT3 downregulates the transcription of several genes involved in immune surveillance: interferon regulatory factor 1 (IRF-1), immunoproteasome subunits (PSM) B8 and B9 and the human leukocyte antigens (HLA). IRF-1 is a PD-L1 inducer; PSMB8-9 and HLA are mediators of effector immune cells activation. STAT1, another member of the STAT family, is activated by JAK upon phosphorylation into dimer conformation and translocates to the nucleus as well. STAT1 has the opposite function of inducing the transcription of IRF-1, PSMB8-9 and HLA. In turn STAT3 can inhibit STAT1 activity. Interferon gamma (IFNγ) is a cytokine released in the tumor microenvironment by the cells of the immune infiltrate. Once binding to its receptor (IFNGR), it triggers signaling cascades that are able to modulate PD-L1 expression. IFNGR can activate both STAT1 and STAT3 through JAK1/2. IFNγ-mediated STAT3 activation also requires SFKs. The alternative activation of either STAT1 or STAT3 is competitive and related to their relative abundance in the tumor cell. IFNGR can also activate cyclin-dependent kinase 5 activator 1 (CDK5R1 or p35), the main CDK5 activator. CDK5 inhibits interferon regulator factor 2 (IRF-2) and IRF2BP2 that are PD-L1 co-repressors.
induces the expression of IRF1, which is a PD-L1 positive regulator.\textsuperscript{56} JAK1/2 mutations have been described as a negative predictive biomarker of response to immune checkpoint blockade, since they prevent the IFN\textgamma-mediated up-regulation of PD-L1.\textsuperscript{57} In another study, JAK1/2 mutations were not related to nivolumab resistance,\textsuperscript{28} which increases the complexity of cancer immune escape. This could be explained by the fact that, in the absence of IFN\textgamma, CKLF-like MARVEL transmembrane domain-containing protein 6 (CMTM6) is a regulator of PD-L1 expression.\textsuperscript{58} CMTM6 can be overexpressed in several types of tumors, including lung cancer.\textsuperscript{59}

\textbf{STAT3 as an immune modulator in oncogene-addicted tumors}

We have shown that early STAT3 activation occurs in EGFR-mutant lung cancer cell lines upon treatment with gefitinib, afatinib or osimertinib, when the drugs are used at 50\% of their inhibitory concentration (IC\textsubscript{50}).\textsuperscript{18,22} We have also found that combined EGFR and STAT3 inhibition can be more effective than EGFR inhibition alone in treatment-naïve and resistant EGFR-mutant NSCLC cell models, \textit{in vitro} and \textit{in vivo}. Moreover, we defined baseline STAT3 mRNA expression as a predictor of outcome to EGFR TKI treatment in EGFR-mutant NSCLC patients.\textsuperscript{18} JAK/STAT3 signaling is a common downstream pathway for many RTKs. In addition, c-Src has an important activity in regulating STAT3 function.\textsuperscript{60} The increased STAT3 activity in EGFR TKI-resistant cells could be mediated by the co-expression of RTKs, other than EGFR.\textsuperscript{61} In castration-resistant prostate cancer, the activation of RTKs, such as, EGFR, AXL (AXL receptor tyrosine kinase) or platelet-derived growth factor receptor a (PDGFRa), could only be abrogated whereas interleukin 1 receptor antagonist (IL-1ra) could only be induced when immune checkpoint blockade was combined with the multi-kinase inhibitor cabozantinib.\textsuperscript{62} IL-1ra reduces MDSCs and increases macrophage polarization.\textsuperscript{62} This synergistic effect can be explained by the multi-kinase inhibitor-mediated suppression of STAT3, facilitating the activity of the immunotherapy. Consistently, there is evidence that STAT3 inhibition is able to reduce MDSC activity in different cancer types.\textsuperscript{63,64} So far there is little evidence, however, on the correlation of STAT3 and immune escape in oncogene-driven tumors. In a melanoma model, STAT3 was found to be mediating the up-regulation of PD-L1 occurring in BRAF inhibitor-resistant cells.\textsuperscript{65}

The expression of PD-L1 in EGFR-mutant or ALK-translocated NSCLC has been widely explored at the preclinical level, as well as in clinical trials. Activated EGFR and ALK induce the expression of PD-L1 in cell line models and cause apoptosis of T cells.\textsuperscript{66,67} Jiang and colleagues explored the correlation between PD-L1 expression and oncogenic alterations such as KRAS, EGFR, MET, ROS1 or ALK, in NSCLC.\textsuperscript{68} PD-L1 protein expression was assessed with the rabbit monoclonal anti-human PD-L1 antibody, clone E1L3N (Cell Signaling Technology, Inc., Denver, MA, USA).\textsuperscript{68,69} They reported that, in lung adenocarcinoma, when using a cutoff of \(\geq5\%\) for PD-L1 positivity, there was a 76\% overlap between PD-L1 positivity and the presence of oncogenic alterations. The overlap was 67\% when the PD-L1 cutoff was \(\geq50\%\).\textsuperscript{68} Overall, they found that PD-L1 was expressed only in 36.5\% and 12.8\% of total lung adenocarcinoma samples, when using a cutoff of \(\geq5\%\) and \(\geq50\%\), respectively,\textsuperscript{68} consistent with the findings of the main clinical trials.\textsuperscript{5,11,70} In the first cohort of the ATLANTIC phase II clinical trial, 72.5\% of EGFR-mutant or ALK-translocated patients had more than 25\% PD-L1 expression.\textsuperscript{71} These data indicate that oncogene-addicted NSCLCs have higher PD-L1 expression than those not driven by an oncogenic alteration.

We recently demonstrated that high PD-L1 mRNA expression is significantly related to better progression-free survival in EGFR-mutant NSCLC treated mainly with gefitinib.\textsuperscript{72} D’Incicco and colleagues previously reported similar data, with PD-L1-positive EGFR-mutant NSCLC patients experiencing better responses to gefitinib or erlotinib in comparison with PD-L1-negative patients.\textsuperscript{73,74} In contrast with EGFR-mutant NSCLC, the predictive role of PD-L1 expression in ALK-translocated tumors is not clear.\textsuperscript{75} Either EGFR or ALK inhibitors down-regulate PD-L1 expression in EGFR-mutant or ALK-translocated human lung cancer cell lines.\textsuperscript{66,67} No synergistic effect was found when EGFR or ALK inhibitors were combined with anti-PD-1/PD-L1 antibodies in co-culture models. Since STAT3 levels can be regulated by RTKs other than EGFR or ALK, we can speculate that the absence of synergism in those models is dependent on STAT3 activation. At the time of resistance to EGFR or ALK inhibitors, the PD-L1 expression is raised again.\textsuperscript{66,67}
EGFR-mutant NSCLC cell lines with MET-mediated acquired resistance to EGFR TKIs, had concomitant up-regulation of PD-L1, which was decreased after treatment with a MET inhibitor.\textsuperscript{76} Considering that the signaling pathways downstream of RTKs are common, we speculate that there is a similar immune modulation pattern in these two subgroups of NSCLC (Figure 1).

In EGFR-mutant NSCLC, STAT3 is directly related to the expression of PD-L1. Specifically, inhibition or silencing of STAT3, or inhibition or knockdown of the driver mutation is able to down-regulate PD-L1 expression in EGFR-mutant or ALK-translocated lung cancer cell lines.\textsuperscript{75,77} However, all three main oncogenic signaling pathways, mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/AKT and STAT3 can be involved in PD-L1 expression. Chen and colleagues have shown that activated EGFR-induced PD-L1 expression was abrogated with a MAPK inhibitor, but not with an AKT inhibitor.\textsuperscript{66} In an EGFR-mutant NSCLC cell line with acquired resistance to gefitinib, PD-L1 expression was down-regulated after STAT3 or AKT, but not after MAPK inhibition.\textsuperscript{78} An increase in STAT3 and AKT phosphorylation, as well as PD-L1 expression, was observed in KRAS-mutant lung cancer cells upon stimulation with IFN\textgamma.\textsuperscript{79} A specific STAT3 or AKT inhibitor could inhibit the IFN\gamma-induced PD-L1 expression in the same model.\textsuperscript{79} Indeed, the activation of STAT3 by IFN\gamma, with subsequent increase in PD-L1 expression, may occur independent of the presence of an oncogenic alteration.\textsuperscript{80} In KRAS-mutant cells, the MAPK pathway is implicated in the regulation of PD-L1 expression.\textsuperscript{81} We believe that signaling pathways common in several types of tumors can differentially regulate immune responses, according to the driver oncogene, with a profound role of STAT3 in EGFR-mutant NSCLC (Figure 1).

**Perspectives**

STAT3 is an immune-response modulator. The role of STAT3 in innate resistance to EGFR TKIs\textsuperscript{18} and the fact that EGFR or ALK inhibition decreases PD-L1 levels, provide the rationale for reconsidering immunotherapy strategies in these two subgroups of NSCLC patients. PD-L1 is high at baseline in oncogene-addicted NSCLC, but monotherapy with immune checkpoint inhibitors has not, to date, demonstrated encouraging results.\textsuperscript{17,82} Although a co-culture model did not show clear synergistic effect,\textsuperscript{66} we believe that a combined approach with TKIs and anti-PD-1/ PD-L1 compounds merits further investigation both in treatment-naïve and after development of acquired resistance to TKIs. Clinical trials are ongoing and phase I studies have shown activity and a good safety profile, with the exception of the combination of durvalumab with osimertinib.\textsuperscript{8} Another strategy that merits to be investigated is the combination of STAT3 inhibitors with immune checkpoint blockade. Simple approaches, like repurposing drugs that affect the tumor microenvironment, can improve the efficacy of immune checkpoint blockade. For instance, pterostilbene, a natural methoxylated analogue of resveratrol, inhibits both STAT3 and Src and prevents the function of T-regulatory lymphocytes (Tregs).\textsuperscript{83} Pentoxifylline, a drug used for chronic occlusive arterial disease, can also prevent the recruitment of Tregs through inhibition of c-Rel, a subunit of the nuclear factor kappa B (NF-\kappa B).\textsuperscript{84} More complex, but with a strong biological rationale, is the concept of combining immune checkpoint blockade with cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors. Similar to STAT3, CDK4 and 6 activate DNMT1 and contribute to an immune-suppressive microenvironment.\textsuperscript{85}

The relation of STAT3 with PD-L1 expression in oncogene-addicted tumors provides evidence that STAT3 can be predictive of response to immunotherapy, and should be further investigated. Given the IFN\gamma-mediated activation of STAT3, and the awareness that this axis is able to up-regulate PD-L1, our model including STAT3 assessment at baseline can indirectly help to define which patients have an active tumor-immune infiltrate to be awakened with anti-PD-1/PD-L1 treatment.

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**Conflict of interest statement**

The authors declare that there is no conflict of interest.
References
1. Horn L, Spigel DR, Vokes EE, et al. Nivolumab versus docetaxel in previously treated patients with advanced non-small-cell lung cancer: two-year outcomes from two randomized, open-label, phase III trials (CheckMate 017 and CheckMate 057). J Clin Oncol 2017; 35: 3924–3933.

2. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015; 373: 123–135.

3. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015; 373: 1627–1639.

4. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet 2017; 389: 255–265.

5. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med 2016; 375: 1823–1833.

6. Chae YK, Oh MS and Giles FJ. Molecular biomarkers of primary and acquired resistance to T-cell-mediated immunotherapy in cancer: landscape, clinical implications, and future directions. Oncologist. Epub ahead of print 14 December 2017. DOI: 10.1634/theoncologist.2017–0354.

7. Wartewig T, Kurgis Z, Keppler S, et al. PD-1 is a haploinsufficient suppressor of T cell lymphomagenesis. Nature 2017; 552: 121–125.

8. Ludin A and Zon LI. Cancer immunotherapy: the dark side of PD-1 receptor inhibition. Nature 2017; 552: 41–42.

9. Attili I, Passaro A, Pavan A, et al. Combination immunotherapy strategies in advanced non-small cell lung cancer (NSCLC): does biological rationale meet clinical needs? Crit Rev Oncol Hematol 2017; 119: 30–39.

10. Perez-Ruiz E, Etxeberria I, Rodriguez-Ruiz ME, et al. Anti-CD137 and PD-1/PD-L1 antibodies en route toward clinical synergy. Clin Cancer Res 2017; 23: 5326–5328.

11. Langer CJ, Gadgeel SM, Borghaei H, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. Lancet Oncol 2016; 17: 1497–1508.

12. Iwai Y, Hamanishi J, Chamoto K, et al. Cancer immunotherapies targeting the PD-1 signaling pathway. J Biomed Sci 2017; 24: 26.

13. Fehrenbacher L, Spira A, Ballinger M, et al. Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): a multicentre, open-label, phase 2 randomised controlled trial. Lancet 2016; 387: 1837–1846.

14. Peters S, Creelan B, Hellman MD, et al. Impact of tumor mutation burden on the efficacy of first-line nivolumab in stage IV or recurrent non-small cell lung cancer: an exploratory analysis of CheckMate 026. In: Proceedings from the 2017 AACR annual meeting, Washington, DC, 1–5 March 2017, Abstract CT082. Cancer Res 2017. DOI: 10.1158/1538-7445.AM2017-CT082.

15. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015; 348: 124–128.

16. Spigel DR, Schrock AB, Fabrizio D, et al. Total mutation burden (TMB) in lung cancer (LC) and relationship with response to PD-1/PD-L1 targeted therapies. J Clin Oncol 2016; 34: 9017–9017.

17. Lee CK, Man J, Lord S, et al. Checkpoint inhibitors in metastatic EGFR-mutated non-small cell lung cancer—a meta-analysis. J Thorac Oncol 2017; 12: 403–407.

18. Chaib I, Karachaliou N, Pilotto S, et al. Co-activation of STAT3 and yes-associated protein 1 (YAP1) pathway in EGFR-mutant NSCLC. J Natl Cancer Inst 2017; 109. DOI: 10.1093/jnci/djx014.

19. Fan W, Tang Z, Yin L, et al. MET-independent lung cancer cells evading EGFR kinase inhibitors are therapeutically susceptible to BH3 mimetic agents. Cancer Res 2011; 71: 4494–4505.

20. Li L, Han R, Xiao H, et al. Metformin sensitizes EGFR-TKI-resistant human lung cancer cells in vitro and in vivo through inhibition of IL-6 signaling and EMT reversal. Clin Cancer Res 2014; 20: 2714–2726.

21. Li R, Hu Z, Sun SY, et al. Niclosamide overcomes acquired resistance to erlotinib through suppression of STAT3 in non-small cell lung cancer. Mol Cancer Ther 2013; 12: 2200–2212.

22. Codony-Servat C, Codony-Servat J, Karachaliou N, et al. Activation of signal transducer and
activator of transcription 3 (STAT3) signaling in EGFR-mutant non-small-cell lung cancer (NSCLC). Oncotarget 2017; 8: 47305–47316.

23. Rosell R, Karachaliou N, Chaib I, et al. Innate resistance in EGFR-mutant non-small cell lung cancer (NSCLC) patients by coactivation of receptor tyrosine kinases (RTKs). Ann Oncol 2017; 28(Suppl. 2): ii1–ii5.

24. Lazzari C, Karachaliou N, Verlicchi A, et al. Molecular bases for combinatorial treatment strategies in KRAS mutant (KRASm) lung adenocarcinoma (LAC). J Thorac Oncol 2016; 11(Suppl. 4): S82.

25. Huynh J, Etemadi N, Hollande F, et al. The JAK/STAT3 axis: a comprehensive drug target for solid malignancies. Semin Cancer Biol 2017; 45: 13–22.

26. Kusaba T, Nakayama T, Yamazumi K, et al. Activation of STAT3 is a marker of poor prognosis in human colorectal cancer. Oncol Rep 2006; 15: 1445–1451.

27. Yakata Y, Nakayama T, Yoshizaki A, et al. Expression of p-STAT3 in human gastric carcinoma: significant correlation in tumour invasion and prognosis. Int J Oncol 2007; 30: 437–442.

28. Riaz N, Havel JJ, Makarov V, et al. Tumor and microenvironment evolution during immunotherapy with nivolumab. Cell 2017; 171: 934–949. e915.

29. Lu C, Talukder A, Savage NM, et al. JAK-STAT-mediated chronic inflammation impairs cytotoxic T lymphocyte activation to decrease anti-PD-1 immunotherapy efficacy in pancreatic cancer. Oncoinmunology 2017; 6: e1299106.

30. Ma C, Horlad H, Pan C, et al. Stat3 inhibitor abrogates the expression of PD-1 ligands on lymphoma cell lines. J Clin Exp Hematop 2017; 57: 21–25.

31. Wolfe SJ, Strebovsky J, Bartz H, et al. PD-L1 expression on tolerogenic APCs is controlled by STAT-3. Eur J Immunol 2011; 41: 413–424.

32. Marzec M, Zhang Q, Goradia A, et al. Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). Proc Natl Acad Sci USA 2008; 105: 20852–20857.

33. Tripathi SC, Peters HL, Taguchi A, et al. Immunoproteasome deficiency is a feature of non-small cell lung cancer with a mesenchymal phenotype and is associated with a poor outcome. Proc Natl Acad Sci USA 2016; 113: e1555–e1564.

34. Ferrington DA and Gregerson DS. Immunoproteasomes: structure, function, and antigen presentation. Prog Mol Biol Transl Sci 2012; 109: 75–112.

35. Zhang X, Blenis J, Li HC, et al. Requirement of serine phosphorylation for formation of STAT-promoter complexes. Science 1995; 267: 1990–1994.

36. Decker T and Kovarik P. Serine phosphorylation of STATs. Oncogene 2000; 19: 2628–2637.

37. Wakahara R, Kunimoto H, Tanino K, et al. Phospho-Ser727 of STAT3 regulates STAT3 activity by enhancing dephosphorylation of phospho-Tyr705 largely through TC45. Genes Cells 2012; 17: 132–145.

38. Lu D, Liu L, Ji X, et al. The phosphatase DUSP2 controls the activity of the transcription activator STAT3 and regulates TH17 differentiation. Nat Immunol 2015; 16: 1263–1273.

39. Yuan X, Dou Y, Wu X, et al. Tetrandrine, an agonist of aryl hydrocarbon receptor, reciprocally modulates the activities of STAT3 and STAT5 to suppress Th17 cell differentiation. J Cell Mol Med 2017; 21: 2172–2183.

40. Hazan-Halevy I, Harris D, Liu Z, et al. STAT3 is constitutively phosphorylated on serine 727 residues, binds DNA, and activates transcription in CLL cells. Blood 2010; 115: 2852–2863.

41. Yu H, Lee H, Herrmann A, et al. Revisiting STAT3 signalling in cancer: new and unexpected biological functions. Nat Rev Cancer 2014; 14: 736–746.

42. Yu H, Kortylewski M and Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol 2007; 7: 41–51.

43. Kortylewski M, Kujawski M, Wang T, et al. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. Nat Med 2005; 11: 1314–1321.

44. Wu L, Du H, Li Y, et al. Signal transducer and activator of transcription 3 (Stat3C) promotes myeloid-derived suppressor cell expansion and immune suppression during lung tumorigenesis. Am J Pathol 2011; 179: 2131–2141.

45. Ferguson SD, Srinivasan VM and Heimberger AB. The role of STAT3 in tumor-mediated immune suppression. J Neurooncol 2015; 123: 385–394.

46. Kortylewski M and Yu H. Role of Stat3 in suppressing anti-tumor immunity. Curr Opin Immunol 2008; 20: 228–233.
47. Nefedova Y, Cheng P, Gilkes D, et al. Activation of dendritic cells via inhibition of Jak2/STAT3 signaling. *J Immunol* 2005; 175: 4338–4346.

48. Nefedova Y, Nagaraj S, Rosenbauer A, et al. Regulation of dendritic cell differentiation and antitumor immune response in cancer by pharmacologic-selective inhibition of the Janus-activated kinase 2/signal transducers and activators of transcription 3 pathway. *Cancer Res* 2005; 65: 9525–9535.

49. Spary LK, Salimu J, Webber JP, et al. Tumor stroma-derived factors skew monocyte to dendritic cell differentiation toward a suppressive CD14+ PD-L1+ phenotype in prostate cancer. *Oncoimmunology* 2014; 3: e955331.

50. Li R, Fang F, Jiang M, et al. STAT3 and NF-kappaB are simultaneously suppressed in dendritic cells in lung cancer. *Sci Rep* 2017; 7: 45395.

51. Zaidi MR and Merlino G. The two faces of interferon-gamma in cancer. *Clin Cancer Res* 2011; 17: 6118–6124.

52. Karachaliou N, Crespo G, Aldeguer E, et al. Interferon-gamma (INFg), an important marker of response to immune checkpoint blockade (ICB) in non-small cell lung cancer (NSCLC) and melanoma patients. *J Clin Oncol* 2017; 35: 11504–11504.

53. Qing Y and Stark GR. Alternative activation of STAT1 and STAT3 in response to interferon-gamma. *J Biol Chem* 2004; 279: 41679–41685.

54. Dorand RD, Nthale J, Myers JT, et al. Cdk5 disruption attenuates tumor PD-L1 expression and promotes antitumor immunity. *Science* 2016; 353: 399–403.

55. Soliman H, Khalil F and Antonia S. PD-L1 expression is increased in a subset of basal type breast cancer cells. *PLoS One* 2014; 9: e88557.

56. Lee SJ, Jang BC, Lee SW, et al. Interferon regulatory factor-1 is prerequisite to the constitutive expression and IFN-gamma-induced upregulation of B7-H1 (CD274). *FEBS Lett* 2006; 580: 755–762.

57. Shin DS, Zaretsky JM, Escuin-Ordinas H, et al. Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. *Cancer Discov* 2017; 7: 188–201.

58. Burr ML, Sparbier CE, Chan YC, et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. *Nature* 2017; 549: 101–105.

59. Mezzadra R, Sun C, Jae LT, et al. Identification of CMTM6 and CMTM4 as PD-L1 protein regulators. *Nature* 2017; 549: 106–110.

60. Garcia R, Bowman TL, Niu G, et al. Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. *Oncogene* 2001; 20: 2499–2513.

61. Yu Y, Luo Y, Zheng Y, et al. Exploring the mechanism of non-small-cell lung cancer cell lines resistant to epidermal growth factor receptor tyrosine kinase inhibitor. *J Cancer Res Ther* 2016; 12: 121–125.

62. Lu X, Horner JW, Paul E, et al. Effective combinatorial immunotherapy for castration-resistant prostate cancer. *Nature* 2017; 543: 728–732.

63. Vasquez-Dunddel D, Pan F, Zeng Q, et al. STAT3 regulates arginase-I in myeloid-derived suppressor cells from cancer patients. *J Clin Invest* 2013; 123: 1580–1589.

64. Hossain DM, Pal SK, Moreira D, et al. TLR9-targeted STAT3 silencing abrogates immunosuppressive activity of myeloid-derived suppressor cells from prostate cancer patients. *Clin Cancer Res* 2015; 21: 3771–3782.

65. Jiang Z, Zhou J, Giobbie-Hurder A, et al. The activation of MAPK in melanoma cells resistant to BRAF inhibition promotes PD-L1 expression that is reversible by MEK and PI3K inhibition. *Clin Cancer Res* 2013; 19: 598–609.

66. Chen N, Fang W, Zhan J, et al. Upregulation of PD-L1 by EGFR activation mediates the immune escape in EGFR-driven NSCLC: implication for optional immune targeted therapy for NSCLC patients with EGFR mutation. *J Thorac Oncol* 2015; 10: 910–923.

67. Hong S, Chen N, Fang W, et al. Upregulation of PD-L1 by EML4-ALK fusion protein mediates the immune escape in ALK positive NSCLC: implication for optional anti-PD-1/PD-L1 immune therapy for ALK-TKIs sensitive and resistant NSCLC patients. *Oncoimmunology* 2016; 5: e1094598.

68. Jiang L, Su X, Zhang T, et al. PD-L1 expression and its relationship with oncogenic drivers in non-small cell lung cancer (NSCLC). *Oncotarget* 2017; 8: 26845–26857.

69. Cogswell J, Inzunza HD, Wu Q, et al. An analytical comparison of Dako 28–8 PharmDx assay and an E1L3N laboratory-developed test in the immunohistochemical detection of programmed death-ligand 1. *Mol Diagn Ther* 2017; 21: 85–93.

70. Rizvi NA, Hellmann MD, Brahmer JR, et al. Nivolumab in combination with platinum-based doublet chemotherapy for first-line treatment of...
advanced non-small-cell lung cancer. *J Clin Oncol* 2016; 34: 2969–2979.

71. Garassino MC, Cho BC, Gray JE, et al. 82O Durvalumab in ≥3rd-line EGFR-mutant/ALK+, locally advanced or metastatic NSCLC: results from the phase 2 ATLANTIC study. *Ann Oncol* 2017; 28(Suppl. 2): ii28–ii51.

72. Karachaliou N, Giménez-Capitán A, Drozdowskyj A, et al. Expression of genes associated with anti-viral response in EGFR-mutant non-small cell lung cancer (NSCLC). *Ann Oncol* 2017; 28(Suppl. 2): ii1–ii5.

73. D’Incecco A, Andreozzi M, Ludovini V, et al. PD-1 and PD-L1 expression in molecularly selected non-small-cell lung cancer patients. *Br J Cancer* 2015; 112: 95–102.

74. Rosell R and Palmero R. PD-L1 expression associated with better response to EGFR tyrosine kinase inhibitors. *Cancer Biol Med* 2015; 12: 71–73.

75. Koh J, Jang JY, Keam B, et al. EML4-ALK enhances programmed cell death-ligand 1 expression in pulmonary adenocarcinoma via hypoxia-inducible factor (HIF)-1alpha and STAT3. *Oncoimmunology* 2015; 5: e1108514.

76. Demuth C, Andersen MN, Jakobsen KR, et al. Increased PD-L1 expression in erlotinib-resistant NSCLC cells with MET gene amplification is reversed upon MET-TKI treatment. *Oncotarget* 2017; 8: 68221–68229.

77. Zhang N, Zeng Y, Du W, et al. The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. *Int J Oncol* 2016; 49: 1360–1368.

78. Abdelhamed S, Ogura K, Yokoyama S, et al. AKT-STAT3 pathway as a downstream target of EGFR signaling to regulate PD-L1 expression on NSCLC cells. *J Cancer* 2016; 7: 1579–1586.

79. Zhang X, Zeng Y, Qu Q, et al. PD-L1 induced by IFN-gamma from tumor-associated macrophages via the JAK/STAT3 and PI3K/AKT signaling pathways promoted progression of lung cancer. *Int J Clin Oncol* 2017; 22(6): 1026–1033.

80. Garcia-Diaz A, Shin DS, Moreno BH, et al. Interferon receptor signaling pathways regulating PD-L1 and PD-L2 expression. *Cell Rep* 2017; 19: 1189–1201.

81. Sumimoto H, Takano A, Teramoto K, et al. RAS-mitogen-activated protein kinase signal is required for enhanced PD-L1 expression in human lung cancers. *PLoS One* 2016; 11: e0166626.

82. Gainor JF, Shaw AT, Sequist LV, et al. EGFR mutations and ALK rearrangements are associated with low response rates to PD-1 pathway blockade in non-small cell lung cancer: a retrospective analysis. *Clin Cancer Res* 2016; 22: 4585–4593.

83. Lee-Chang C, Bodogai M, Martin-Montalvo A, et al. Inhibition of breast cancer metastasis by resveratrol-mediated inactivation of tumor-evoked regulatory B cells. *J Immunol* 2013; 191: 4141–4151.

84. Grinberg-Bleyer Y, Oh H, Desrichard A, et al. NF-kappaB c-Rel is crucial for the regulatory T cell immune checkpoint in cancer. *Cell* 2017; 170: 1096–1108. e1013.

85. Goel S, DeCristo MJ, Watt AC, et al. CDK4/6 inhibition triggers anti-tumour immunity. *Nature* 2017; 548: 471–475.