

# Tumor Genotype Is Shaping Immunophenotype and Responses to Immune Checkpoint Inhibitors in Solid Tumors

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## ABSTRACT

A major breakthrough in cancer treatment was ushered in by the development of immune checkpoint blockade therapy such as anti-CTLA4 antibody and anti-PD-1 and anti-programmed cell death-ligand 1 antibodies that are now approved for use in an increasing number of malignancies. Despite the relative success of immune checkpoint inhibitors with certain tumor types, many patients still fail to respond to such therapies, and the field is actively trying to understand the mechanisms of resistance, intrinsic or acquired, to immune checkpoint blockade. Herein, we discuss the roles that somatic genomic mutations in oncogenic pathways play in immune editing, as well as some of the current approaches toward improving response to immunotherapy.

**Keywords:** immunotherapy, PTEN, EGFR, angiogenesis

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## INTRODUCTION

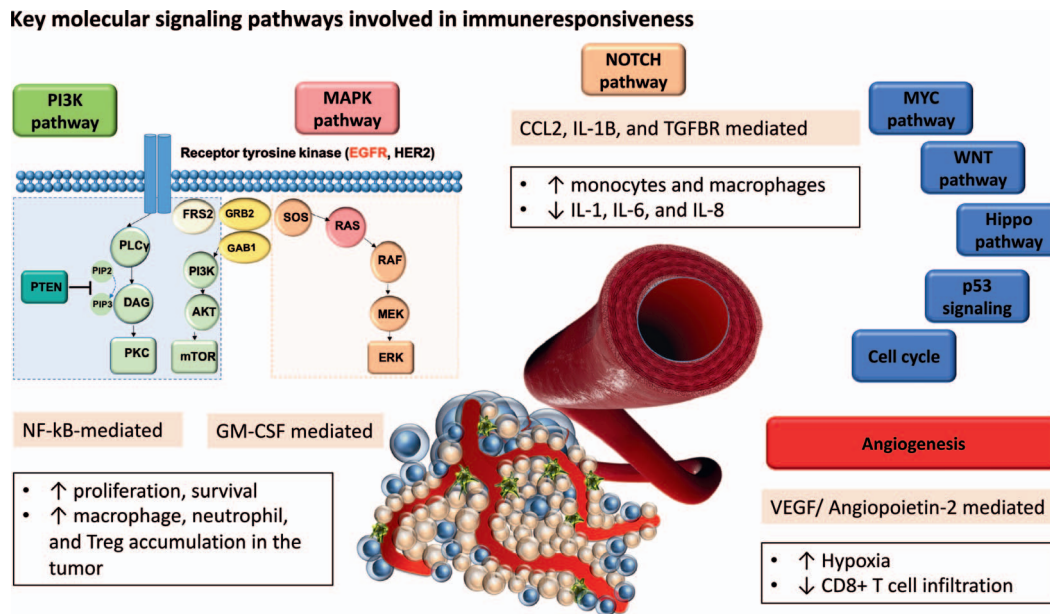
Immune checkpoint inhibitors such as anti-PD-1, anti-programmed cell death-ligand 1 (PD-L1), and anti-CTLA4 antibodies, work by activating dormant T cells directed at cancer cells and, since their development, have revolutionized cancer treatment in the past decade for multiple tumor types.<sup>[1,2]</sup> However, it remains uncertain why only a small percentage of patients develop long responses to these treatments.<sup>[3]</sup> There are likely several factors that could influence cancer immune responsiveness, including tumor immune microenvironment, somatic alterations, germline variants, and transcriptional changes.<sup>[4]</sup> An example of direct relation between tumor genomic alterations and response to immunotherapy are the somatic mutations in mismatch repair genes (MMR) and high microsatellite instability (MSI-H), leading to a particular immunophenotype with increased responsiveness to immune checkpoint inhibitors.<sup>[5]</sup> Increased tumor mutation burden (TMB), usually defined as the number of nonsynonymous mutations per megabase of DNA sequenced, is also a predictive biomarker for better response to PD-1 blockade and improved clinical outcomes.<sup>[6,7]</sup>

With the integration of next-generation sequencing in the clinic for tumor molecular profiling for personalized cancer treatment, there is an increasing knowledge about the somatic alterations that could influence the response to immunotherapy.<sup>[8]</sup>

Here we review how somatic genomic alterations in key oncogenic pathways and angiogenesis are shaping immune editing, and we discuss current approaches to improve immune responsiveness and to personalize immunotherapy treatments for cancer patients (Fig. 1 and Table 1). We performed a literature search on PubMed to identify key biomarkers contributing to immune checkpoint inhibitor resistance. We limited our search results to recent data, after 2015, and to actionable alterations for which targeted therapies are either approved or are currently in clinical trials.

## Angiogenesis

One of the hallmarks of tumor growth and progression is new vessel formation.<sup>[9,10]</sup> Once it reaches a critical size, the metabolic needs of the growing tumor can no longer be sustained by the existing vasculature, thereby triggering the “angiogenic switch.”<sup>[11]</sup> During this process, pro-angiogenic factors such as vascular endo-



**Figure 1.**—Key oncogenic and angiogenic pathways involved in immune editing. PI3K: phosphoinositide 3-kinase; EGFR: Epidermal Growth Factor Receptor; HER2: human epidermal growth factor receptor 2; PIP2: phosphatidylinositol 4,5-bisphosphate; PIP3: phosphatidylinositol (3,4,5)-trisphosphate; PLC $\gamma$ : phospholipase gamma; DAG: diacylglycerol; PKC: protein kinase C; FRS2: fibroblast growth factor receptor substrate 2; AKT: Ak strain transforming (AKT) serine/threonine kinase, or protein kinase B; mTOR: mammalian target of rapamycin; GRB2: growth factor receptor-bound protein 2; GAB1: GRB2-associated-binding protein 1; SOS: son of sevenless; RAS: rat sarcoma; RAF: rapidly accelerated fibrosarcoma; MEK: MAPK/ERK kinase; ERK: extracellular signal-regulated kinases; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; GM-CSF: granulocyte-macrophage colony-stimulating factor; IL-1 $\beta$ : interleukin-1 $\beta$ ; TGF $\beta$ R: transforming growth factor beta receptor; MYC: myelocytoma proto-oncogene; WNT: wingless-type MMTV integration site family; p53: tumor protein p53.

thelial growth factor (VEGF), platelet-derived growth factor, angiopoietins (ANGPT), and interleukin 8 (IL-8) overcome antiangiogenic factors such as angiostatin and endostatin, tipping the imbalance toward tumor neovascularization.<sup>[12,13]</sup> The resulting tumor vasculature is leaky and disorganized, further exacerbating the problem by oversupplying certain portions of the tumor with nutrients and oxygen while undersupplying others. Furthermore, this dysfunctional vascular network impacts the efficiency of delivery of therapeutic agents to cancer cells.<sup>[14]</sup>

How the immune system regulates angiogenesis is still not completely understood; however, there is increasing evidence that endothelial cells lining the tumor vasculature can assist in the escape of cancer cells from immunosurveillance.<sup>[15]</sup> Inhibitory molecules, including PD-L1, PD-L2, indoleamine 2,3-dioxygenase (IDO1), the T-cell immunoglobulin domain, and mucin domain protein 3 (TIM3), are expressed by the endothelium and contribute to the regulation of immune cell recruitment, adhesion, and function.<sup>[16–19]</sup> In several tumor types, including breast, prostate, and colon cancers, endothelial cells also selectively express the Fas ligand (FasL).<sup>[20]</sup> This so called “tumor endothelial death barrier” allows the killing of effector T cells, but not of regulatory T cells (Treg) through tumor-derived VEGF, IL-10, and prostaglandin action.<sup>[21]</sup> Tumor endothelial cells also present tumor antigens to T cells, resulting in their activation.<sup>[22]</sup> Expression of costimulatory molecules CD80 and CD86, which are required for activation of naïve T cells, has also been reported for endothelial cells.<sup>[23]</sup>

In an analysis of biopsies from melanoma patients obtained prior to receiving immune checkpoint blockade therapies, treatment-resistant tumors displayed a transcriptional signature that indicated the upregulated expression of angiogenic genes (*VEGFA*, *VEGFC*, *FLT1*, and *ANGPT2*). Certain subsets of T cells also produce transforming growth factor- $\beta$  (TGF $\beta$ ), which stimulates regulatory T cells and further contributes to angiogenesis and immunosuppression.<sup>[24]</sup> Tumor-associated macrophages have also been found to promote angiogenesis and influence responses to immunotherapy.<sup>[25]</sup>

A preclinical study using prostate and melanoma mouse models has demonstrated that activation of the tumor endothelium with tumor necrosis factor (TNF) has promoted intratumoral T-cell infiltration, depletion of regulatory T cells, and expansion of cytotoxic T lymphocytes, all of which contribute to the overall therapeutic activity of immune checkpoint inhibitors.<sup>[26]</sup>

It has also been shown that endothelial cells, rather than the metastatic renal tumor cells themselves, are responsible for increased IDO expression and that IDO-1 expression levels in the endothelium could also be predictive of response to immunotherapy.<sup>[27–30]</sup>

In a preclinical study, immune checkpoint inhibition increased tumor vessel perfusion by promoting CD8+ T-cell accumulation and interferon (IFN)- $\gamma$  production in treatment-sensitive breast and colon tumor cell lines, but not in treatment-resistant models.<sup>[31,32]</sup> The stimulator of IFN genes (STING) has been shown to have antitumor activity, and it is expressed also on endothelial cell vasculatures, suggesting that the combination of STING

**Table 1.**—Summary of angiogenic and oncogenic pathways implicated in immune editing

Gene	Mechanism	References
Angiogenesis	Expression of FasL allows selective killing of effector T cells through tumor-derived VEGF, IL-10, and prostaglandin action. Expression of molecules such as IDO and STING by endothelial cells is associated with response or overcoming resistance to immunotherapy.	Motz et al. <sup>[20]</sup> Carman et al. <sup>[21]</sup> Seeber et al. <sup>[27]</sup> Chevolet et al. <sup>[28]</sup> , Krähenbühl et al. <sup>[29]</sup> Meireson et al. <sup>[30]</sup> Yang et al. <sup>[33]</sup>
<i>EGFR</i> Mutation/ Overexpression	Aberrant expression leads to decreased PD-L1 expression in NSCLC and is associated with decreased tumor mutational burden and immunogenicity. Activating mutations associated with increased PD-L1 and immunosuppressive cytokine expression, and reduced T-cell viability.	Dong et al. <sup>[45]</sup> , Soo et al. <sup>[46]</sup> , Zhang et al. <sup>[49]</sup> , Gainor et al. <sup>[52]</sup> Chen et al. <sup>[39]</sup> , Akbay et al. <sup>[40]</sup> , Azuma et al. <sup>[41]</sup>
<i>PTEN</i> Loss	Loss leads to increased PD-L1 expression, increased autologous T-cell apoptosis, and overall decreased tumor immunogenicity. <i>PTEN</i> loss leads to increased immunosuppressive cytokine expression (including VEGF), decreased T-cell trafficking, and inhibition of autophagy and cytolytic activity.	Parsa et al. <sup>[65]</sup> ; Waldron et al. <sup>[66]</sup> Peng et al. <sup>[67]</sup> ; George et al. <sup>[69]</sup>
<i>NOTCH</i> Amplification	Promotes an immunosuppressive microenvironment by increasing myeloid-derived suppressor cells, tumor-associated macrophages and regulatory T cells, and decreasing cytotoxic T cells. Activation of the <i>NOTCH</i> pathway promotes TH1 and inhibits TH2 differentiation. Promotes TGF $\beta$ signaling-mediated tumor growth through upregulation of proinflammatory IL1 $\beta$ and CCL2 cytokines and recruitment of tumor-associated macrophages.	Shen et al. <sup>[78]</sup> Balli et al. <sup>[79]</sup> Qiu et al. <sup>[80]</sup> Tindemans et al. <sup>[81]</sup> Shen et al. <sup>[78]</sup>

FasL: Fas ligand; VEGF: vascular endothelial growth factor; IL-10: interleukin-10; IDO: indoleamine 2,3-dioxygenase; STING: stimulator of interferon genes; PD-L1: programmed death-ligand 1; NSCLC: non-small cell lung cancer; *PTEN*: phosphatase and tensin homolog; TH1: T helper type 1; TH2: T helper type 2; TGF $\beta$ : transforming growth factor beta; IL1 $\beta$ : interleukin 1 beta; CCL2: C-C motif chemokine ligand 2.

agonists with anti-PD1 or anti-PD-L1 antibodies and antiangiogenics could overcome primary or secondary resistance to immunotherapy.<sup>[33]</sup>

Numerous studies have already explored efforts to normalize the tumor vasculature to improve responses to immune checkpoint blockade.<sup>[34–36]</sup>

The coadministration of antiangiogenic agents can allow for the transient improvement of the tumor vasculature and result in enhanced drug delivery and immune cell infiltration, as well as improved response to immunotherapy. This hypothesis was clinically proven by the recent Keynote-426 trial, showing improved overall and progression-free survival (PFS) in patients with metastatic renal cell carcinoma treated with axitinib and pembrolizumab when compared to the sunitinib group.<sup>[37]</sup> Furthermore, the combination of lenvatinib (a multityrosine kinase inhibitor targeting VEGF receptors 1-3 [VEGFR-1-3], fibroblast growth factor receptors 1-4 [FGFR-1-4], RET, c-kit) and pembrolizumab showed 39% objective responses in endometrial cancer and was recently FDA approved for this indication.<sup>[38]</sup>

### Epidermal Growth Factor Receptor (EGFR) Mutations and Activation of the Mitogen-Activated Protein Kinase (MAPK) Pathway Signaling

Activating mutations in *EGFR* lead to downstream signaling through the MAPK pathway and has been associated with increased expression of PD-L1.<sup>[39]</sup> Upreg-

ulation of PD-L1 in *EGFR*-mutant non-small cell lung cancer (NSCLC) cells cocultured with human peripheral blood mononuclear cells has led to reduced T-cell viability, increased expression of immune checkpoint molecules, and immunosuppressive cytokines.<sup>[40]</sup> Preclinical studies have shown that PD-1 inhibition improved survival of mice with *EGFR*-driven adenocarcinomas by enhancing effector T-cell function and also reduced viability of NSCLC tumors with aberrant *EGFR* expression.<sup>[41]</sup> Taken together, these studies imply that *EGFR*-activating mutations contribute to an immunosuppressive tumor microenvironment and that patients with *EGFR* mutations may not respond favorably to anti-PD-1 or anti-PD-L1 therapy.

In a randomized, open-label phase 3 study, NSCLC patients receiving nivolumab had improved overall survival (OS) and PFS compared to those who received docetaxel, with the exception of those who had never smoked or had *EGFR* mutations.<sup>[42]</sup> In another phase 2/3 study, patients with NSCLC with at least 50% of tumor cells expressing PD-L1 had significantly longer OS and PFS with pembrolizumab than with docetaxel.<sup>[43]</sup> This includes those with *EGFR*-mutant status, although the authors do acknowledge the lower incidence of *EGFR* mutations encountered in this study than was expected in the general NSCLC population. Two other studies that performed meta-analyses of multiple immunotherapy trials compared to standard chemotherapy demonstrated that only *EGFR*-wild-type patients benefit from anti-PD1

or anti-PD-L1 antibodies and that patients with *EGFR*-mutated tumors did not achieve improved OS or longer PFS while on immune checkpoint inhibitor therapy.<sup>[44]</sup>

In contrast to the studies described earlier, epidemiologic studies suggest an inverse relationship between oncogenic *EGFR* mutations and PD-L1 expression.<sup>[45]</sup> A pooled analysis of close to 4000 patients from 19 peer-reviewed studies demonstrated that aberrant oncogenic *EGFR* expression in NSCLC was less likely to be PD-L1-positive compared to wild-type tumors.<sup>[46]</sup> Two other epidemiologic reports show that patients with activating *EGFR* mutations have a higher likelihood of decreased PD-L1 expression and that increased PD-L1 expression was actually associated with wild-type *EGFR*.<sup>[47,48]</sup>

Although there is mechanistic evidence supporting increased PD-L1 expression associated with *EGFR*-activating mutations, the concept of adaptive immune resistance renders support to an inverse relationship between *EGFR* and PD-L1 that could be mediated by the IL-6/JAK/STAT3<sup>[49]</sup> or NF $\kappa$ B<sup>[50]</sup> pathways. Cancer cells are capable of increasing PD-L1 expression in response to a robust immune attack that is usually mounted by tumor antigen-specific T cells. This process is largely dependent on effective immune recognition, which itself is dependent on increased somatic mutational and neoantigen burden. *EGFR*-driven tumors have been reported to possess lower mutational burden.<sup>[51]</sup>

Quantitative pooled analysis of the TCGA (The Cancer Genome Atlas) and Broad Institute dataset has shown that tumors with oncogenic *EGFR* mutations, specifically those with *EGFR*-activating mutations, have a significantly reduced tumor mutational burden and lower immunogenicity compared to those that are wild type.<sup>[45]</sup> This is consistent with a study by Gainor and colleagues,<sup>[52]</sup> which not only reported that *EGFR*-mutant NSCLCs have lower PD-L1 expression compared to their wild-type counterparts but also that the lower overall response to PD-1/PD-L1 inhibition may be due to the reduced infiltration of CD8+ lymphocytes in this tumor microenvironment.

In addition to *EGFR*, some alterations that activate the MAPK pathway (Fig. 1), such as KRAS G12D, can confer immune resistance.<sup>[53-55]</sup> On the other hand, recent findings have also suggested that KRAS G12C in these tumor types might likely have a different immune profile and may respond better to immune checkpoint inhibitors.<sup>[56,57]</sup> This pathway, in addition to the known oncogenic roles, also has important functions in CD4 T-cell differentiation, IL-4 receptor function, and IL-10 and IL-12 production regulation.<sup>[58,59]</sup>

### Phosphatase and Tensin Homolog (PTEN) Deletions and Inactivating Mutations

PTEN is a lipid phosphatase that acts as the primary negative regulator of intracellular phosphoinositide 3-kinase (PI3K), a major downstream effector of receptor tyrosine kinases and G protein-coupled receptors. In response to receptor binding by growth factors, PI3K

generates phospholipids, which in turn activate Akt (serine/threonine kinase) and other downstream effectors involved in cellular processes such as cell survival, proliferation, and differentiation.<sup>[60]</sup> Components of the PI3K pathway (such as *PIK3CA* and *PTEN*) are frequently mutated in cancer; *PTEN* itself is considered a tumor suppressor since loss of *PTEN* expression due to deletions or inactivating mutations results in increased tumor cell survival and growth.<sup>[61]</sup>

*PTEN* alterations are frequently observed in many tumor types, including melanoma, glioblastoma, prostate, endometrial, and breast cancers.<sup>[62,63]</sup> Frequent loss of at least one copy of *PTEN* has been reported in human pancreatic ductal adenocarcinoma (PDAC).<sup>[64]</sup> Experiments in mice revealed activation of the PI3K pathway resulting from *PTEN* loss not only leads to PDAC development but also influences the tumor microenvironment by promoting NF- $\kappa$ B activation, thereby favoring tumor growth. *PTEN* loss and subsequent activation of the PI3K pathway in human glioblastoma tumors have also been reported to increase PD-L1 expression. Moreover, human glioma cells expressing wild-type *PTEN* were demonstrated to be more sensitive to T-cell-mediated lysis in vitro compared to *PTEN* mutant gliomas.<sup>[65]</sup> Tumors with loss of *PTEN* expression thus are poorly immunogenic, with immune resistance mediated in part by PD-L1 expression. In a follow-up study, *PTEN* wild-type gliomas elicited minimal T-cell apoptosis compared to tumors deficient in *PTEN* when cocultured with autologous T cells. Inhibition of the PI3K/AKT pathway in tissues lacking *PTEN* expression restored tumor-induced T-cell apoptosis to wild-type levels, providing mechanistic in vitro evidence for the involvement of this pathway to immune resistance following *PTEN* loss.<sup>[66]</sup>

Peng et al.<sup>[67]</sup> demonstrated that silencing of *PTEN* in melanoma cells results in reduced T-cell-mediated cytolytic activity. Clinical specimens from melanoma patients likewise demonstrated correlation of *PTEN* loss with decreased T-cell tumor infiltration and reduced expansion of tumor-derived T cells. Moreover, metastatic melanoma patients with *PTEN*-expressing tumors displayed better responses to FDA-approved checkpoint inhibitors than did their *PTEN*-deficient counterparts. Loss of *PTEN* expression was also shown to contribute to increased immunosuppressive cytokine production and subsequent inhibition of autophagy. Although the exact mechanism needs to be further defined, taken together, these suggest that *PTEN* loss leads to resistance to immunotherapy in melanoma by decreasing both T-cell trafficking into tumors and T-cell-mediated cell death in a PD-L1 independent manner.<sup>[68]</sup> A study of an exceptional uterine leiomyosarcoma responder on anti-PD-1 (pembrolizumab) monotherapy also reported that biallelic *PTEN* loss, along with reduced expression of two neoantigens, are potential mediators of resistance to immune checkpoint inhibitors. This study also reported

increased VEGF expression with decreased T-cell infiltration in the treatment-resistant, *PTEN*-deficient tumor.<sup>[69]</sup>

## NOTCH Signaling Pathway

NOTCH signaling, highly conserved through evolution, has important functions in regulating developmental processes and tissue homeostasis.<sup>[70]</sup> Activation of the NOTCH pathway occurs after a NOTCH receptor (NOTCH 1-4) is bound to one of the Delta-like ligands (DLL 1-4) or Jagged ligands (Jag 1,2),<sup>[71]</sup> and subsequently the activated NOTCH intracellular domain translocates in the nucleus where it activates the transcription of genes involved in tumor growth and proliferation.<sup>[72]</sup>

NOTCH signaling an important role in T-cell acute lymphoblastic leukemia (T-ALL), where *NOTCH1*-activating mutations are one of most frequent alterations in this disease.<sup>[73]</sup> In addition to T-ALL, NOTCH signaling also has oncogenic functions in other tumor types such as breast, colorectal, and ovarian cancers, NSCLC, medulloblastoma, and melanoma.<sup>[71,74,75]</sup> Furthermore, depending on the cell type, alterations in *NOTCH1* can act as a tumor suppressor.<sup>[76,77]</sup>

NOTCH1 signaling has been shown to increase the myeloid-derived suppressor cells, tumor-associated macrophages, and regulatory T cells, and to decreased cytotoxic T cells with a role in promoting an immunosuppressive microenvironment in several tumor types such as breast cancer, melanoma, and pancreatic cancer.<sup>[78–80]</sup> Signaling through the NOTCH pathway in CD4+ T cells has an important role in promoting T helper type 1 (TH1) and inhibiting TH2 differentiation.<sup>[81]</sup>

In basal-like breast cancer, Notch activation promotes tumor growth through TGF $\beta$  signaling by proinflammatory cytokines such as IL-1 $\beta$  and chemokine ligand 2 (CCL2) and recruitment of tumor-associated macrophages.<sup>[78]</sup>

Targeting the NOTCH pathway has been done mostly by gamma-secretase inhibitors, but other antibodies targeting NOTCH receptors or ligands are currently in development.

Because of NOTCH signaling implications in TH population differentiation and its promotion of immunosuppressive tumor microenvironment, it provides a rationale for possible combination studies of NOTCH pathway-targeted therapies with immune checkpoint inhibitors or other T-cell therapies.<sup>[82]</sup>

However, a major concern in treatments involving gamma-secretase inhibitors (GSI) with other agents has been gastrointestinal toxicities, warranting alternate therapeutic strategies to overcome this.<sup>[83]</sup>

## CONCLUSIONS

Dysregulation of key signaling pathways that promote tumorigenesis can also influence the immunosurveillance. The influence of tumor genomics on the activation of the tumor immune microenvironment can

explain the heterogenous response to immunotherapy among the same tumor type. There is an acute need to identify biomarkers that could help personalize immunotherapy and identify patients who are more or less likely to benefit for immunotherapy inhibitors. Although high microsatellite instability by mutations in MMR genes and high TMB are becoming validated biomarkers for responsiveness to immunotherapy inhibitors, these do not explain all responses to immunotherapy inhibitors. Identifying actionable alterations in key signaling molecular pathways that have immunomodulatory effect could help the development of genomically matched treatments and help personalize immunotherapy inhibitors.

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