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Integrating immunotherapy and targeted therapy in cancer treatment: mechanistic insights and clinical implications

Johann S. Bergholz[1,2,3,*], **Qiwei Wang**[1,2,*], **Sheheryar Kabraji**[1,4], **Jean J. Zhao**[1,2,3]

[1]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, USA

[2]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

[3]Broad Institute of Harvard and MIT, Cambridge, MA, USA

[4]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA

Abstract

Small molecule targeted therapies have demonstrated outstanding potential in the clinic. These drugs are designed to minimize adverse effects by selectively attacking cancer cells while exerting minimal damage to normal cells. Although initial response to targeted therapies may be high, yielding positive response rates and often improving survival for an important percentage of patients, resistance often limits long-term effectiveness. On the other hand, immunotherapy has demonstrated durable results, yet for a limited number of patients. Growing evidence indicates that some targeted agents can modulate different components of the anti-tumor immune response. These include immune sensitization by inhibiting tumor cell-intrinsic immune evasion programs or enhancing antigenicity, as well as direct effects on immune effector and immunosuppressive cells. The combination of these two approaches, therefore, has the potential to result in synergistic and durable outcomes for patients. In this review, we focus on the latest advances on integrating immunotherapy with small molecule targeted inhibitors. In particular, we discuss how specific oncogenic events differentially affect immune response, and the implications of these findings on the rational design of effective combinations of immunotherapy and targeted therapies.

Introduction

Over the past decade, immunotherapy has cemented its status as a vital component of cancer care. In particular, immune checkpoint blockade (ICB) agents, most notably inhibitors of the PD-1/PD-L1 and CTLA-4 pathways, have become standard of care for many solid and

Corresponding authors: Jean J. Zhao, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, Phone: 617.632.2932, jean_zhao@dfci.harvard.edu; Johann S. Bergholz, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215, Phone: 617.582.7523, johann_bergholz@dfci.harvard.edu.

*These authors contributed equally to this work

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J.S.B. is a scientific consultant for Geode Therapeutics. J.S.B. and J.J.Z. are co-inventors of DFCI 2180.001 (DFS-166.25). Q.W. is a scientific consultant for Crimson Biopharm. Q.W. and J.J.Z. are co-inventors of DFCI 2409.001 (DFS-203.60). J.J.Z. is a founder and director of Crimson Biopharm and Geode Therapeutics.

hematologic malignancies, leading to durable results and improved long-term protection from relapse. This latter effect is likely mediated by the induction of an adaptive immune memory capable of eradicating otherwise obstinate tumor cells. Despite their broad applicability, however, ICB benefits only a limited number of patients. Best durable responses have been observed in melanoma, where five-year survival was reported at 26% for ipilimumab (anti-CTLA-4) and 44% for nivolumab (anti-PD-1), and non-small cell lung cancer (NSCLC), where overall survival (OS) approximates 16% after five years (1,2). In an effort to harness this unique potential, the research field has seen a revamped focus on understanding how current and new therapies can influence anti-tumor immune response. In fact, multiple strategies aiming to potentiate immunotherapy are currently under preclinical and clinical investigation. Conventional cancer therapies and small molecule targeted inhibitors have been shown to modulate various components of tumor immunity and response to immunotherapy (3–5). Targeted agents in particular exert these effects by altering mechanisms of immune escape encoded by oncogenic pathways in tumor cells. Therefore, a deeper understanding of how specific oncogenic events shape tumor immunity will prove crucial to the successful development of immunomodulatory strategies. To this end, targeted therapies are ideally situated to block or enhance relevant pathways while exerting minimal damage to normal cells.

Immune evasion by cancer cells

The process by which tumor cells evade immune surveillance can be better understood through the concept of immunoediting (1,6). Initially, malignant cells are regularly detected and eliminated by the immune system through recognition of immunogenic antigens and generation of an innate and adaptive immune response. Acute inflammation activates innate immunity, leading to dendritic cell (DC) maturation and subsequent priming of T-cells, which are central to the anti-tumor response. This constant pressure, however, may act to select for tumor cells that are able to escape immune attack and remain in equilibrium until further changes promote overt tumor growth. This final process is usually accompanied by a shift from acute to chronic inflammation and the establishment of an immunosuppressive tumor microenvironment (TME) via recruitment of immune suppressive cells whose normal function is to dampen immune response, including regulatory T-cells (T_{Regs}), pro-tumorigenic tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs), which further facilitate tumor growth. As a consequence, T-cells in general become exhausted or dysfunctional, and therefore unable to mount an effective anti-tumor response (Fig. 1).

Oncogenic signaling pathways shape the tumor immune microenvironment

Oncogenic signaling pathways have the potential to affect every component of tumor immunity. Careful analysis of clinical studies and the development of relevant animal models are key steps to maximize translational potential. Studies using genetically engineered mouse models (GEMMs) correlated with clinical data have provided much insight into how specific oncogenic events differentially contribute to immune escape. Mechanistic studies for target prediction and biomarker discovery, as well as pre-clinical evaluation in mouse models thus provide important information for designing potentially

successful clinical trials (Fig. 2). Importantly, mechanisms of immune evasion and de novo as well as acquired resistance to immunotherapy often overlap, thus underscoring the potential for targeted approaches that could simultaneously sensitize tumors to immunotherapy and prevent recurrence. In this section, we review some of the major molecular mechanisms described to date.

MYC

The MYC oncogene was shown to directly up-regulate expression of the innate immune inhibitor receptor CD47, a so called “don’t eat me” signal, and of the adaptive immune checkpoint ligand PD-L1 in lymphoma/leukemia models of conditional MYC over-expression (Fig. 3A) (7). These results were subsequently corroborated by multiple groups in different cancer models (8). Furthermore, conditional MYC activation in a $KRAS^{G12D}$-driven model of lung cancer showed that MYC drives tumor progression and recruitment of an immunosuppressive TME characterized by a marked influx of macrophages and depletion of T-cells, B-cells and natural killer (NK) cells (9). These effects were mediated by tumor-secreted CCL9 and IL-23, which enhanced recruitment of PD-L1+ macrophages and promoted lymphocyte exclusion, respectively (Fig. 3A) (9). In turn, MYC deactivation reversed these changes and led to tumor regression, which was dependent on NK cells but not on T-cells (9). Notably, CCL9/IL-23 co-blockade inhibited tumor progression, while PD-L1 blockade restored T-cell infiltration but did not measurably affect tumor growth (9). More recently, newly developed small molecule MYC inhibitors that disrupt MYC/MAX dimerization were shown to promote anti-tumor immune response, and to synergistically inhibit tumor growth of MyC-Cap mouse prostate cancer allografts when combined with PD-1 blockade (10).

KRAS

$KRAS^{G12D}$ was shown to mediate immune suppression in a GEMM of colorectal carcinoma (CRC) with inducible $KRAS^{G12D}$ and additional APC and p53 double deletion (11). In this case, $KRAS^{G12D}$ repressed expression of IRF2, thus alleviating repression of CXCL3 expression by CRC tumor cells and promoting recruitment of CXCR2+ MDSCs to the TME (Fig. 3B) (11). While single agents against PD-1 or CXCR2 did not affect tumor growth or survival, combined treatment significantly increased survival and inhibited tumor growth (11). Furthermore, a novel $KRAS^{G12C}$-specific inhibitor, AMG510, strongly promoted a pro-inflammatory TME and synergized with anti-PD-1 to inhibit mouse syngeneic CT-26 CRC tumors with enforced $KRAS^{G12C}$ expression, which led to complete regression in 90% of cases (9/10) and immunological memory, as shown by the ability to reject a second challenge of CT-26 tumor cells (12).

EGFR and HER2

Mutant EGFR in lung cancer mouse models has been shown to promote the establishment of an immunosuppressive TME characterized by low levels of cytotoxic T lymphocytes (CTLs) and increased markers of T-cell exhaustion (Fig. 3C) (13). Ectopic mutant EGFR expression in bronchial epithelial BEAS2B cells up-regulates PD-L1 expression, while small molecule EGFR inhibition in NSCLC cell lines down-regulates PD-L1 (13). Consistently, mouse lung adenocarcinoma tumors driven by $Egfr^{L858R}$ display high myeloid cells infiltration, reduced

CD4+ T-helper response and blunted CD8+ T-cell expansion, compared to tumors driven by *Kras*^{G12D} or concomitant *Kras*^{G12D} and p53 deletion (14). In the case of HER2, HER2-positive breast cancers predominantly exhibit immune subtypes consistent with ongoing immune activity, including IFNγ-dominant phenotype (~50% of cases; characterized by strong CD8+ and anti-tumorigenic macrophage signals) and wound healing phenotype (~44% of cases; characterized by high expression of angiogenic genes, high proliferation and T_H2-type responses) (15,16). In addition to inhibiting oncogenic HER2 signaling in tumor cells, anti-HER2 targeted monoclonal antibodies stimulate innate and adaptive immune responses critical for clinical efficacy (17). These effects are mediated primarily via antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis, and by inducing antigen cross-presentation and T-cell priming (17). Considering the aggressive nature of HER2+ breast cancers and the outstanding therapeutic effect of anti-HER2 monoclonal antibodies, these observations underscore the power of the immune system to subdue highly malignant tumor cells.

PTEN

Genetic loss of PTEN is associated with reduced anti-tumor immunity in multiple cancers (18–20). In melanoma, PTEN deficiency correlated with decreased response to ICB in a cohort of patients (n = 39), and with decreased immune activation scores in melanoma samples from TCGA (20). Interestingly, PTEN deficiency and WNT/β-catenin pathway activation were largely non-overlapping (20). Using a BRAF-mutant melanoma xenograft model with ectopic expression of melanoma antigen gp100 and MHC class I H2-D^b, which is specifically recognized by CD8+ T-cells from transgenic PMEL-1 mice, it was shown that PTEN silencing in tumor cells reduced T-cell infiltration and cytotoxic response (Fig. 3D) (20). Moreover, since PTEN-deficient tumors preferentially signal through PI3Kβ (21), treatment with the PI3Kβ isoform-specific inhibitor GSK2636771 improved response to PD-1 blockade in a GEMM of $BRAF^{V600E}$/PTEN-null melanoma (20). Similarly, a novel chimeric GEMM of metastatic castration-resistant prostate cancer (mCRPC) with triple deletion of PTEN, p53 and Smad4 showed markedly enhanced response to combined PD-1/CTLA-4 blockade when combined with GSK2636771 (22). These mCRPC tumors were highly infiltrated by Gr-MDSCs, which contributed to primary resistance to immunotherapy, and showed synergistic response to ICB in combination with targeted agents that preferentially affect Gr-MDSCs, such as the tyrosine kinase inhibitor cabozantinib, the PI3K/mTOR dual inhibitor BEZ235 and the CXCR1/2 inhibitor SX-682 (22). Of note, the same group had previously reported that additional loss of Smad4 in a PTEN-null prostate cancer GEMM dramatically enhances tumor progression, metastatic spread and lethality (23), and up-regulates CXCL5 expression in tumor cells via HIPPO-YAP1 signaling, which enhances recruitment of immune suppressive CXCR2+ MDSCs (22).

WNT/β-catenin

Analysis of human melanoma samples revealed a correlation between T-cell exclusion and WNT/β-catenin signaling, including gain-of-function mutations on the β-catenin gene (*CTNNB1*) and up-regulated expression of β-catenin target genes (24). To further investigate these findings, the authors compared a GEMM of metastatic melanoma driven by $BRAF^{V600E}$ and PTEN loss with a syngeneic model harboring additional constitutively

active β-catenin, thus showing that β-catenin inhibits production of CCL4 by tumors cells, which leads to impaired recruitment of CD103+ DCs and consequently to impaired T-cell activation (Fig. 3E) (24). Of note, while $BRAF^{V600E}$/PTEN-null tumors responded to combined PD-L1/CTLA-4 blockade and exhibited significant growth inhibition, tumors with additional β-catenin activation failed to respond to this immunotherapy (24). Consistently, WNT/β-catenin signaling was found to inversely correlate with T-cell infiltration in colorectal cancer (25), and across multiple cancer types compiled from The Cancer Genome Atlas (TCGA) (26).

LKB1

Loss of LKB1 in a mouse model of NSCLC driven by mutant KRAS results in neutrophil accumulation and increased T-cell exhaustion (27). Interestingly, LKB1 loss is associated with decreased PD-L1 expression and resistance to PD-1 blockade in mouse models and patient tumors (27). Indeed, retrospective analyses of clinical response in patients with KRAS-mutant lung adenocarcinoma identified genomic mutations on LKB1 as a significant biomarker for primary resistance to anti-PD-1/PD-L1 immunotherapy, as well as in another cohort of NSCLC irrespective of KRAS status (28). Further work demonstrated that LKB1 deficiency in KRAS-mutant lung cancer results in down-regulation of STING and, consequently, an inability to respond to cytoplasmic double-stranded DNA (dsDNA) (29). STING down-regulation facilitates immune escape by preventing STING-mediated expression of type I interferons (IFNs) and pro-inflammatory cytokines, which are necessary for proper engagement and activation of anti-tumor immune response (Fig. 3F) (30).

STAT3 and NF-κB

Signaling pathways that regulate expression of inflammatory cytokines, such as STAT3 and NF-κB, have the potential to dramatically affect immune response. STAT3 can promote immune escape by up-regulating immune suppressive genes, including IL-6, IL-10, TGFβ and VEGF, while simultaneously down-regulating immune effector genes such as type I and II IFNs, IL-12, CD80, CD86, MHC class II molecules, CCL5 and CXCL10 (31). Tumor cell-intrinsic STAT3 promotes paracrine activation of STAT3 in various populations of immune cells, thereby reducing NK and T-cell cytotoxicity, inhibiting DC maturation and T_H1-type response, and stimulating immunosuppressive cells such as MDSCs, T_{Regs} and TAMs (Fig. 3G) (32,33). In a breast cancer GEMM driven by the polyoma virus middle T antigen (PyMT), which is characterized by aggressive and metastatic tumors with latencies around three to four weeks and 80% penetrance, genetic ablation of *Stat3* resulted in early hyperplastic lesions that were readily cleared by the immune system, although after a latency averaging 40 weeks, 30% of these mice developed non-metastatic tumors that escaped immune surveillance and markedly lacked immune infiltration (34). In addition, STAT3 inhibits expression of numerous immunostimulatory genes downstream of NF-κB (31). The NF-κB pathway plays an important role in activating programs of immune response; however, aberrant NF-κB signaling has been shown to exert strong oncogenic effects by up-regulating genes that promote cell proliferation and survival (35). STAT3 binding to NF-κB promotes transactivation of oncogenic genes and prevents binding to genes involved in immune response (31,36). Furthermore, multiple upstream events, including growth factor and cytokine receptors, non-receptor tyrosine kinases like Src and Abl, and Toll-like

receptors (TLRs) induce STAT3 and NF-κB activation either directly, or indirectly via autocrine and paracrine signaling (31).

NOTCH

Dysregulated NOTCH signaling in tumor cells can up-regulate expression of anti-inflammatory cytokines, including TGFβ, IL-4, IL-6 and IL-10, thereby promoting an immunosuppressive TME (Fig. 3H) (37).

FAK

Focal Adhesion Kinase (FAK) was shown to induce CD8+ T-cell exhaustion and promote T_{Reg} recruitment via regulation of multiple cytokines, including CCL1/5/7, CXCL10 and TGFβ2, in a mouse model of squamous cell carcinoma (SCC) (Fig. 3I), and these effects could be reversed by pharmacological targeting of FAK by VS-4718 (38). Similar findings were described in pancreatic ductal adenocarcinoma (PDAC), where FAK inhibition with VS-4718 renders $Kras^{G12D}$; $Trp53^{L/+}$ PDAC tumors sensitive to adoptive cell transfer (ACT) or PD-1 blockade immunotherapy (39).

Integrating small molecule targeted therapy and immunotherapy to improve therapeutic outcomes

Distinct small molecule targeted therapies have been shown to exert specific effects on anti-tumor immune response in mouse models and in the clinic (Fig. 4A). Inhibitors of BRAF, Cyclin-dependent kinase 4 and 6 (CDK4/6) and Poly (ADP-ribose) polymerase 1/2 (PARP) are currently being tested in combination with ICB in clinical trials and have thus far shown promising potential. In this section we discuss these three kinds of inhibitors as examples of targeted agents with immune modulatory properties.

BRAF inhibitors

Treatment with BRAF inhibitors has been shown to increase melanoma differentiation antigen (MDA) expression and presentation by tumor cells, increase NK cell infiltration, and reduce T_{Reg} and MDSC levels in cell and mouse models of BRAF-mutant melanoma (Fig. 4B) (40–42). Using the SM1 model of $BRAF^{V600E}$ mouse melanoma and SM1 cells stably expressing the chicken ovalbumin (OVA) antigen (SM1-OVA), treatment with the BRAF inhibitor vemurafenib improved ACT immunotherapy with T-cells specific against OVA as well as with PMLE-1 T-cells recognizing endogenous gp100 in SM1 cells (43). Furthermore, BRAF inhibition with dabrafenib in combination with the MEK inhibitor trametinib enhanced PMLE-1 ACT, leading to increased CD8+ T-cell infiltration and cytotoxicity, and complete tumor regressions (44). Combined dabrafenib and trametinib also improved response to PD-1 blockade in this model (44). Analysis of biopsy samples from patients with metastatic melanoma also revealed an association between treatment with combined BRAF and MEK inhibition, and increased MDA expression and CD8+ T-cell infiltration (45). More recently, results from a randomized phase 2 clinical trial of combined dabrafenib, trametinib and PD-1 blockade by pembrolizumab compared to dabrafenib, trametinib and placebo showed encouraging results, including improved progression-free

survival and enhanced response, although the triple combination also resulted in increased adverse effects (46,47).

CDK4/6 inhibitors

CDK4/6 inhibitors exert direct immune-stimulatory effects on both tumor and immune cells (Fig. 4C). In tumor cells, the CDK4/6 inhibitors palbociclib and abemaciclib were shown to down-regulate expression of the DNA methyltransferase DNMT1, leading to decreased methylation and subsequently increased expression of endogenous retrovirus (ERV) elements, thus stimulating production of type III IFNs, and a consequent increase in antigen presentation and enhanced CD8+ T-cell effector function (48). Moreover, CDK4/6 inhibition specifically inhibited *ex vivo* proliferation of CD4+ CD25+ T_{Regs}, but did not affect proliferation of CD4+ CD25− and CD8+ T-cells (48). Splenic CD4+ FOXP3+ T_{Reg} levels were also decreased upon treatment *in vivo* independently of the presence of a tumor (48). PD-L1 inhibition significantly improved response to abemaciclib in an inducible GEMM of HER2+ breast cancer, and resulted in complete tumor regression of CT-26 CRC tumors in all cases, as well as the ability to reject a second challenge with CT-26 tumor cells (48). In addition, an *in vitro* small molecule screen identified CDK4/6 inhibitors as capable of directly enhancing T-cell activation via up-regulation of NFAT signaling, a family of transcription factors that are required for proper activation and function of T-cells (49). Consistently, CDK4/6 inhibition by palbociclib or trilaciclib potentiated PD-1 blockade to stimulate anti-tumor T-cell function and inhibit tumor growth in the MC38 and CT-26 CRC models (49). Interestingly, Cyclin D-CDK4 was shown to promote PD-L1 proteasomal degradation (50). *In vivo* treatment with CDK4/6 inhibitors increased tumor PD-L1 levels and sensitized CT-26 tumors to ICB, resulting in complete tumor regression in 67% (8/12) of mice receiving combined palbociclib and anti-PD-1 (50). A study of 348 ER+/HER2− tumor samples collected from patients prior to start of CDK4/6 inhibitor treatment with palbociclib, ribociclib or abemaciclib revealed *FAT1* deletion as a mechanism of therapeutic resistance (51). Mechanistically, *FAT1* loss resulted in engagement of the Hippo pathway, leading to YAP/TAZ translocation to the nucleus and up-regulation of CDK6 expression (51). In the clinic, preliminary results from a phase Ib clinical trial of combined abemaciclib and pembrolizumab in ER-positive/HER2-negative metastatic breast cancer have shown safety profiles similar to single agents and an initial objective response rate (ORR) of 14.3% (52).

PARP inhibitors

Recent studies have demonstrated that, in addition to direct cytotoxicity, the therapeutic efficacy of PARP inhibitors (PARPi) requires coordinated activation of robust local and systemic anti-tumor immune response, such as increased infiltration of effector $CD4^+$ and $CD8^+$ T-cells into the TME, increased intratumoral DCs with potent antigen-presentation capacity, and systemic reduction of MDSCs in tumor, spleen and blood (53,54). Mechanistically, dsDNA derived from homologous recombination (HR)-deficient tumor cells upon PARP inhibition activates cGAS/STING in tumor cells and/or DCs to drive a cGAS/STING-dependent type I IFN signal that mediates antitumor immunity (Fig. 4D) (53). This mechanism of PARPi-triggered STING-dependent antitumor immunity has been demonstrated in several cancer types, including ovarian cancer, triple-negative breast cancer

(TNBC), and lung cancer (53–57). Interestingly, PARP inhibitors have also been shown to induce expression of PD-L1 in tumor cells via multiple mechanisms, including as a response to interferon expression, inactivation of GSK3β, reduced poly(ADP-ribosyl)ation with concomitantly increased phosphorylation of STAT3, and STING activation (56,58–62). While PARPi-mediated PD-L1 up-regulation can promote adaptative immune suppression, it can be overcome by ICB. Indeed, pre-clinical studies have shown that PD-1/PD-L1 blockade further augments PARPi-triggered immune response, leading to more durable suppression of tumor growth and prolonged survival (53–56). Combined PARP inhibition and ICB is being evaluated by numerous clinical trials in first-line, maintenance, and recurrent settings of both HR-deficient and HR-proficient solid tumors (63–68). In general, these trials have found combination therapies are well-tolerated, with safety concerns consistent with individual agent profiles, and have produced encouraging initial results. While PARP inhibition and PD-1/PD-L1 monotherapy exhibit low efficacy for patients with platinum-resistant ovarian cancer who lack a *BRCA* mutation, with ORRs approximately 5% and 4-10%, respectively (69–74), in the ongoing phase I/II TOPACIO/KEYNOTE-162 trial, combined niraparib plus pembrolizumab demonstrated improved efficacy (ORR, 19%) in *BRCA* wild type patients with recurrent platinum-resistant ovarian cancer (75).

Future prospects

It is clear that tumor cell-intrinsic signaling mechanisms strongly affect immune composition and function. A deeper understanding of these molecular and cellular mechanisms will not only help in the design of potentially promising clinical trials of combination therapies targeted to specific groups of patients, but will also help discover new therapeutic targets with previously unknown functions in tumor immunity. Nevertheless, clinical development may still be limited by lack of significant benefit and compounding adverse effects. Careful pre-clinical and clinical studies are needed to improve the efficacy and tolerability of targeted therapy and immunotherapy combinations. Some areas of focus should include the need to: 1) better understand tissue-specific oncogene-related immune effects; 2) identify and validate biomarkers to predict response and resistance to oncogene targeting; 3) develop high fidelity animal models incorporating patient-derived tumors and humanized immune systems to better identify effective combinations without causing increased toxicity to patients; and 4) use multiplexed assays to integrate immune and tumor intrinsic molecular changes in response to combination therapy. Still, current evidence from pre-clinical and clinical trials is in aggregate promising and encouraging. The notion that specific targeted agents can sensitize tumor cells to immunotherapy, thereby leading to durable and effective responses in patients that would otherwise not respond is worth pursuing. Continued basic and pre-clinical research integrated with careful clinical trial planning of combination therapies will likely continue to yield meaningful treatment options for patients afflicted by cancer.

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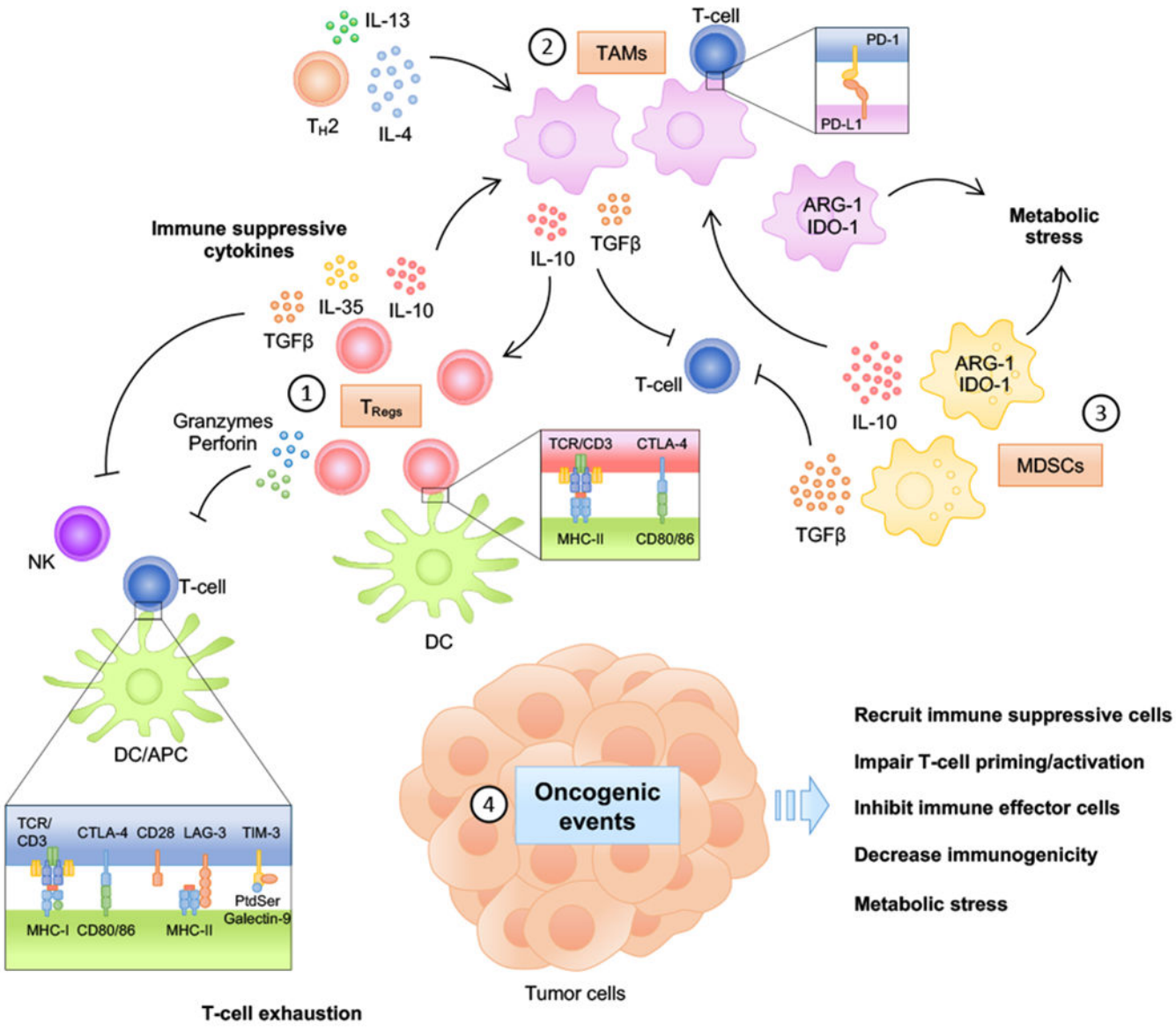

Figure 1.

Generation of an immune suppressive tumor microenvironment. **(1)** Regulatory T-cells (T_{Regs}) suppress immune response via direct cell contact and humoral mechanisms. T_{Regs} constitutively express CTLA-4, which binds to CD80 and CD86 on antigen-presenting cells, such as dendritic cells (DCs), leading to impaired DC maturation and blocking binding of CD80/CD86 to CD28 on conventional T-cells, thereby preventing co-stimulation and T-cell activation. Moreover, T_{Regs} can directly target effector T-cells (T_{Eff}) and natural killer (NK) cells for destruction by secreting cytotoxic granzymes and perforin. Secretion of inhibitory cytokines such as TGFβ, IL-10 and IL-35 further inhibit anti-tumor immune response. **(2)** Tumor-associated macrophages (TAMs) are a major component of the immune infiltrate in solid tumors. Chronic inflammation within the TME and production of IL-4 and IL-13 by T_H2 cells and IL-10 by T_{Regs} induce pro-tumorigenic macrophage polarization. In turn, TAMs exacerbate immune suppression by releasing cytokines such as IL-10 and TGFβ that

suppress T_{Eff} and NK cells but stimulate T_{Regs}. Pro-tumorigenic TAMs also up-regulate metabolic enzymes such as IDO-1 and Arg-1, which can severely affect the composition of the immune infiltrate by competing for catabolism of nutrients. In addition, TAMs can directly inhibit T-cells by expressing immune checkpoint ligands PD-L1 and PD-L2. **(3)** Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells that accumulate in response to chronic inflammation and fail to differentiate into mature cells. MDSCs secrete significant levels of IL-10 and TGFβ, thereby inducing T_{Reg} accumulation and pro-tumorigenic macrophage polarization, while simultaneously inhibiting T_{Eff} and NK cells function and activation. Furthermore, MDSCs promote metabolic stress by dramatically depleting nutrients needed for T-cell function. **(4)** Oncogenic events can directly and indirectly inhibit immune response by multiple mechanisms: a) Numerous cytokines secreted by tumor cells, including TGFβ, IL-10 and the pro-angiogenic molecule VEGF, promote recruitment of immune suppressive cells. b) Down-regulation of pro-inflammatory chemokines, including CCL3, CCL4 and CCL5, and CXCR3 ligands such as CXCL9 and CXCL10 result in decreased DC and T-cell recruitment and impaired T-cell priming/activation. c) Expression of PD-L1 and direct inhibition of T-cell effector function by tumor cells has been observed in numerous cancer types. PD-L1 can be induced by multiple non-exclusive mechanisms, including by cytokines such as type I and II IFNs, TNFα and IL-10, and specific oncogenic events, including chromosomal amplification and up-regulation by oncogenic signaling. d) Decreased immunogenicity may result from defects in antigen presentation and/or defective response to IFNγ, which can occur due to genomic inactivation or downregulation of class I MHC and MHC-related molecules (e.g. B2M) or of genes related to the IFNγ pathway. e) Tumor cells also exert strong metabolic stress on the immune infiltrate by competing for nutrients and secreting byproducts that negatively affect immune effector function. (Reviewed on (1,4,6).)

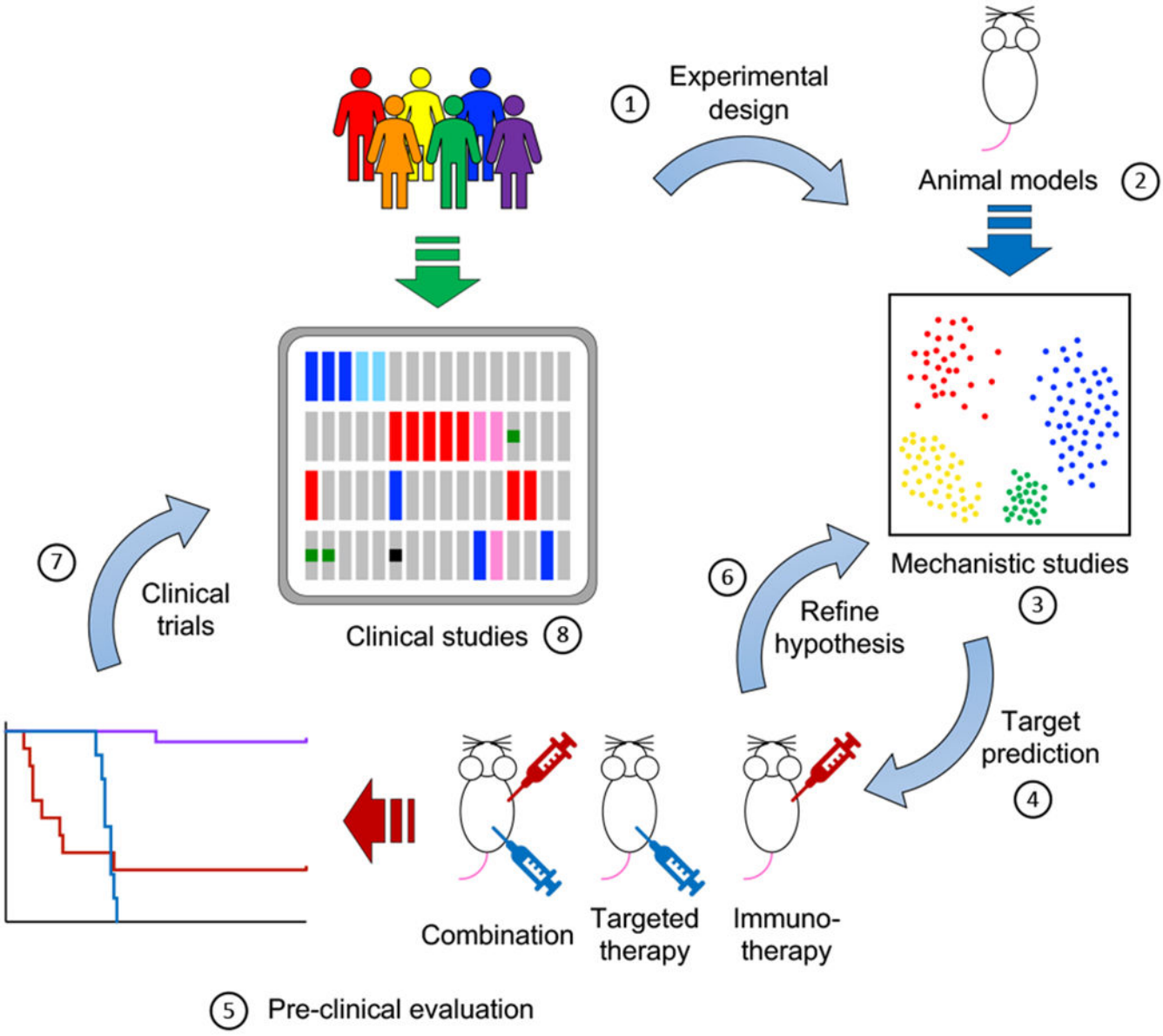

Figure 2.

Integration of clinical and animal studies in translational immuno-oncology. **(1)** Experimental design informed by clinical observations to maximize translational potential. **(2)** Development of animal models that recapitulate genetic abnormalities found in the clinic. In this regard, immunocompetent, syngeneic mouse models provide the current gold standard. Emerging technologies, such as mouse models engrafted with humanized immune systems can improve the clinical relevance of pre-clinical studies and maximize translational feasibility. **(3)** Mechanistic studies are a crucial component of modern tumor immunology research. Complementing classical gain- and loss-of-function experiments, powerful technologies such as single-cell RNA sequencing (scRNA-Seq), cytometry by time-of-flight (CyTOF) and highly multiplex tissue cyclic immunofluorescence (t-CyCIF) have greatly enhanced our ability to interrogate the nature and degree of interplay between tumor and

immune cells. Future work will undoubtedly entail more robust integration of single-cell expression analyses with single-cell spatial relationships within tissues. **(4-6)** Iterative rounds of target prediction **(4)**, pre-clinical evaluation **(5)** and refining hypothesis **(6)** are needed to identify promising targets of clinical relevance. **(7)** Results from pre-clinical studies are used to inform and support the design of clinical trials for promising combinations.

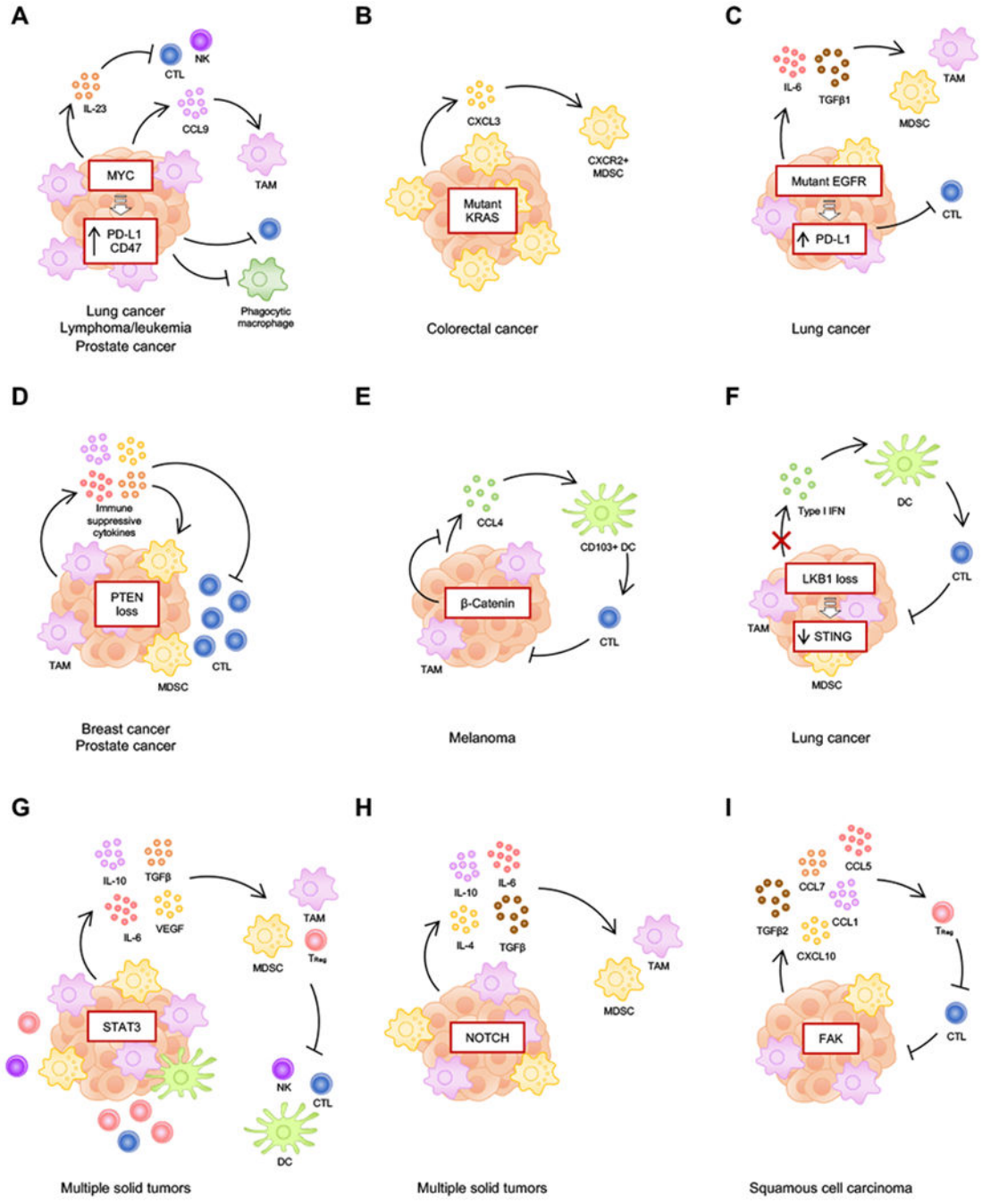

Figure 3.

Tumor-intrinsic molecular mechanisms of immune suppression driven by specific oncogenic events. Selected examples are depicted based on mechanistic studies on animal models. Of note, co-occurring oncogenic events affect immune suppressive mechanisms, thus increasing immune heterogeneity between cancer cases. Additional mechanisms linked to each example have also been described but could not be included in this diagram due to space constraints. **A**, MYC has been shown to promote T-cell and natural killer (NK) cell exclusion, and infiltration of tumor-associated macrophages (TAMs), while also directly

inhibiting T-cells and phagocytic macrophages via upregulation of PD-L1 and CD47. **B**, Mutant KRas has been shown to promote recruitment of myeloid-derived suppressor cells (MDSCs) to the TME through upregulation of CXCL3. **C**, Mutant EGFR has been shown to up-regulate PD-L1 in tumor cells and to induce recruitment of TAMs and MDSCs. **D**, Loss of PTEN is associated with increased production of immune suppressive cytokines, which promote the establishment of an immune suppressive tumor microenvironment (TME) and inhibit T-cell infiltration. **E**, β-Catenin has been shown to inhibit secretion of CCL4 by tumor cells, hence preventing activation of CD103+ dendritic cells (DCs) and subsequent cytotoxic T lymphocyte (CTL) activation. **F**, Loss of LKB1 down-regulates the STING pathway in tumor cells, thereby preventing release of type I IFNs in response to cytoplasmic double-stranded DNA (dsDNA), which would otherwise stimulate immune response. **G**, STAT3 signaling in tumor cells induces upregulation of multiple cytokines that contribute to the establishment of an immune suppressive TME by stimulating suppressive immune cells and inhibiting effector cells. **H**, Dysregulated NOTCH promotes an immune suppressive TME via multiple anti-inflammatory cytokines. **I**, FAK has been shown to stimulate regulatory T-cells (T_{Regs}) by upregulating numerous cytokines.

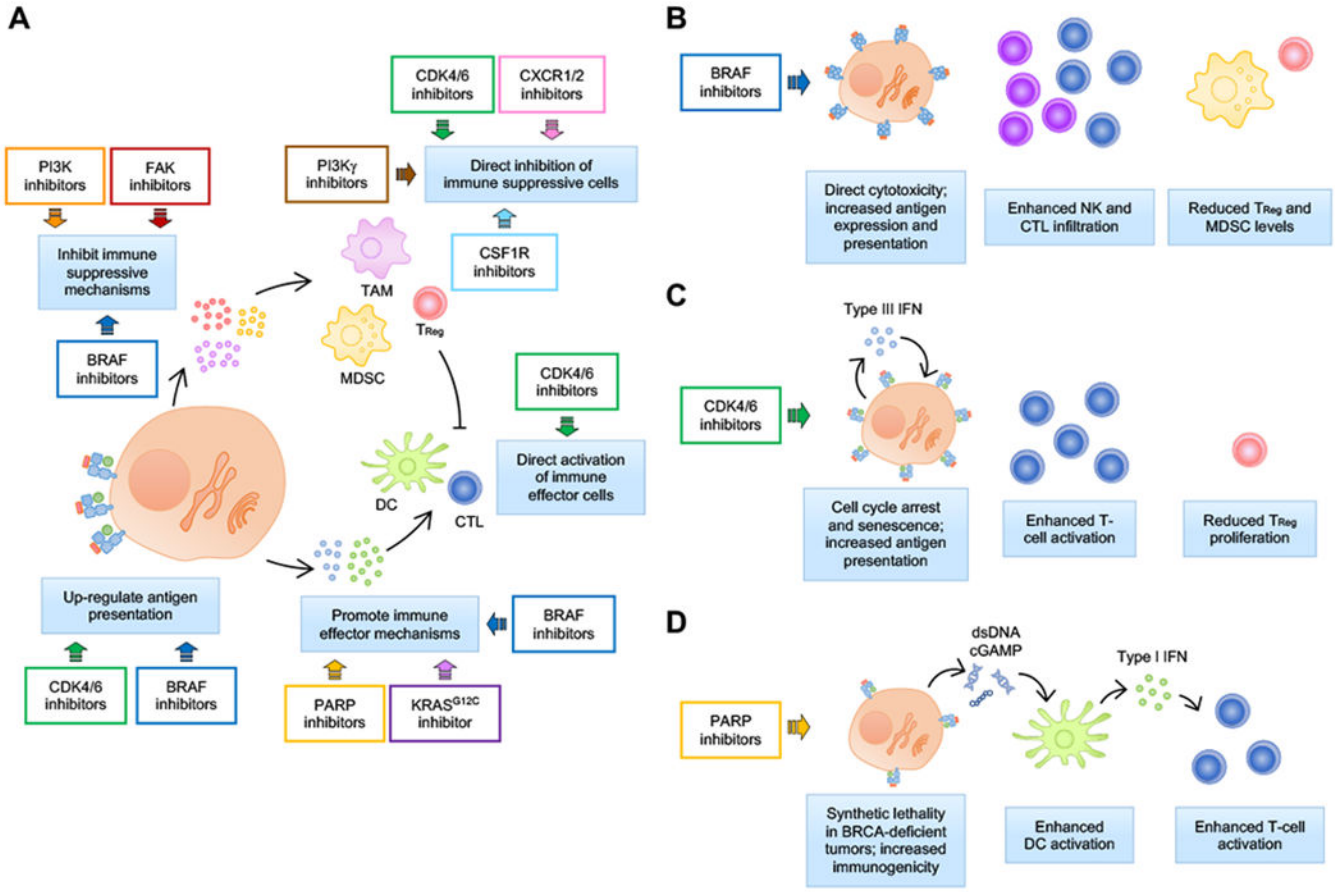

Figure 4.

Immune modulation by small molecule targeted therapies. **A**, Targeted therapies have been shown to affect multiple aspects of cancer immunity, including inhibition of anti-inflammatory mechanisms and promotion of pro-inflammatory mechanisms, up-regulation of antigen presentation, and direct modulatory effects on immune cells. Some targeted agents have been designed to specifically target immune sub-populations. For example, PI3Kγ and CSFR1 inhibitors are used to deplete tumor-associated macrophages (TAMs), and CXCR1/2 inhibitors are used to inhibit myeloid-derived suppressor cells (MDSCs). Other drugs, such as BRAF, PI3K, FAK and $KRAS^{G12C}$ inhibitors, were found to affect immune-related mechanisms in addition to their intended cytotoxic effect on tumor cells largely because oncogenic signaling from tumor cells modulates immune response. In the case of PARP inhibitors, enhanced immunogenicity seems to be a corollary of its primary effect on inducing irreparable DNA damage; however, engagement of a robust immune response is required for effective response. And in the case of CDK4/6 inhibitors, unexpected effects in tumor antigenicity as well as direct effects on immune suppressive and immune effector cells have been reported by independent research groups. **B-D**, Summary of immune modulatory effect of selected examples of targeted therapies currently under clinical investigation in combination with immune checkpoint blockade immunotherapy.