

# Chromatin regulation and immune escape PBAF chromatin remodeling complex deficiency enhances tumor immunotherapy

Ehsan Ghorani and Sergio A Quezada

Cancer Immunology Unit, Research Department of Haematology, University College London Cancer Institute, University College London, UK.  
Email: s.quezada@ucl.ac.uk

Antigens expressed by cancer cells target them for elimination by tumor-infiltrating T cells (1). But, despite T cell recognition, advanced malignancies are often fatally progressive. The discovery of T cell inhibitory (checkpoint) receptors, including programmed cell death protein 1 (PD1) and cytotoxic T lymphocyte-associated protein 4 (CTLA4), that contribute to immune suppression and dysfunction in tumors has led to the advent of checkpoint inhibitors (CPIs) to block these pathways and derepress T cell activity. This has considerably improved outcomes for various cancer types. However, beyond certain rare and highly sensitive tumors (2), responses remain limited to a fraction of patients, and both primary and acquired resistance are frequently observed. Although much work has focused on defining and overcoming T cell-intrinsic inhibitory mechanisms, such as checkpoint expression, less is known about what regulates tumor cell sensitivity to T cell attack. On pages XXX and ZZZ of this issue, Miao *et al.* (3) and Pan *et al.* (4), respectively, find that chromatin remodeling pathways contribute to cancer cell immune resistance through control of interferon-stimulated gene (ISG) expression. This has implications for our understanding of why CPIs fail and suggests that targeting these pathways may enhance tumor immunotherapy.

The interferons (IFNs) are a group of cytokines with antitumor effects, mediated by activation of Janus kinases (JAKs) and signal transducers and activators of transcription (STATs). STATs bind ISG promoters to drive transcription and thus gene expression. ISGs promote programmed cell death and antiproliferative effects in addition to enhancing tumor cell immunogenicity by increasing the expression of tumor antigen processing and presentation machinery. Thus, ISG expression is usually tumor suppressive. Of the three types of IFN, type II or IFN- $\gamma$  is a key effector of antitumor T cell function with its tumor suppressive effects mediated predominantly through activation of STAT1 and the expression of a subset of ISGs. Deficient IFN- $\gamma$  signaling enhances tumorigenesis in mice, and loss-of-function mutations in downstream signaling pathways are well characterized in tumor cell lines and tumor tissues (5). In melanoma, inactivating mutations of genes that encode members of these pathways are enriched in CPI-resistant tumors (6).

STAT binding to ISG promoters is regulated by multiprotein complexes that remodel chromatin to control DNA accessibility. The switch/sucrose nonfermentable (SWI/SNF) family consists of BRG1- or hBRM-associated factors (BAF) and polybromo-BAF (PBAF) subgroups with distinct roles in regulating transcriptional programs driving a broad range of cellular processes. Although sharing a core set of subunits, PBAF complexes are distinguished by the inclusion of bromodomain-containing protein 7 (BRD7), AT-rich interactive domain-containing protein 2 (ARID2), and polybromo 1 (PBRM1) (7). SWI/SNF complex genes are commonly inactivated through mutation in ~20% of human cancers. Specifically, *PBRM1* is mutated in ~40% of patients with clear cell renal cell cancer (ccRCC) and acts as a tumor suppressor gene with roles in DNA repair, maintenance of genome stability, and control of cell proliferation (8).

Miao *et al.* and Pan *et al.* validate a role for IFN- $\gamma$  signaling in cancer cell sensitivity to antitumor immune responses and further characterize chromatin remodeling as a regulator of this. Miao *et al.* found that truncating loss-of-function mutations in *PBRM1* were significantly enriched in ccRCC patients who respond to anti-PD1 or anti-PD1 ligand 1 (PDL1) CPIs. Exploring the potential transcriptional consequences of PBAF loss (owing to PBRM1 mutation), the authors found that immune-stimulatory ISGs were strongly expressed in the absence of PBAF. Moreover, they observed an intriguing increase in the expression of genes downstream of STAT3 and STAT5. In addition to driving oncogenic gene transcription, both STAT3 and STAT5 (9) interfere with STAT1 mediated antiproliferative effects of IFN- $\gamma$  signaling, suggesting that these factors are induced to counterbalance the tumor-suppressive effects of STAT1.

Using a CRISPR screen in a CPI-resistant mouse melanoma cell line, Pan *et al.* confirmed that genes involved in antigen presentation and IFN- $\gamma$  signaling are important for immune sensitivity. All three PBAF-specific genes (*BRD7*, *ARID2*, and *PBRM1*) were identified in the screen, suggesting a role in conferring resistance to T cell attack. Gene expression and chromatin accessibility analyses of PBAF-deficient cells revealed increased ISG transcription in concordance with greater accessibility of DNA at target promoters. *Pbmr1*-deficient melanomas in mice had greater T cell infiltration and sensitivity to combined anti-PD1 plus anti-CTLA4 CPIs, in agreement with the findings of Miao *et al.*

Together with recently published CRISPR screens that similarly attribute immune resistance to genes involved in IFN- $\gamma$  signaling (10), these studies add further *in vivo* and clinical data to support the importance of the IFN- $\gamma$  pathway in the response to CPIs. The remaining key question is what other tumor cell-intrinsic pathways play a role in CPI resistance? Tumor metabolic properties are emerging as potential regulators of immune activity, and putative resistance genes in these pathways were found in the screen of Pan *et al.* The contribution of these pathways to control of immune susceptibility is not well characterized, and further work is required to understand this.

SWI/SNF complexes have previously been implicated in enhancing ISG transcription (11). By what mechanisms may loss of PBAF function have the same effect? The epigenetic silencer polycomb repressive complex 2 (PRC2) is overexpressed in cancer cells and mediates repression of multiple IFN- $\gamma$ -stimulated genes (12). The recent demonstration that PBRM1 cooperates with the PRC2 subunit enhancer of zeste homolog 2 (EZH2) to promote silencing of ISGs (13) suggests a possible mechanism to explain why PBAF loss is associated with the induction of ISG expression, but further work is required to validate this (see the figure).

Given the tumor suppressive effects of PBAF, the finding that inactivating mutations in PBAF subunits sensitize cancer cells to T cell-mediated destruction suggests a trade-off between concurrently enhanced tumorigenicity and tumor suppressive immunogenicity. The finding by Miao *et al.* that PBAF deficiency is associated with increased STAT3 and STAT5 signaling suggests the deployment of cell-intrinsic mechanisms to counteract mutant PBAF-mediated enhancement of immune susceptibility. Agents with activity against these STATs are either approved or in development (14) and may additionally synergize with CPIs.

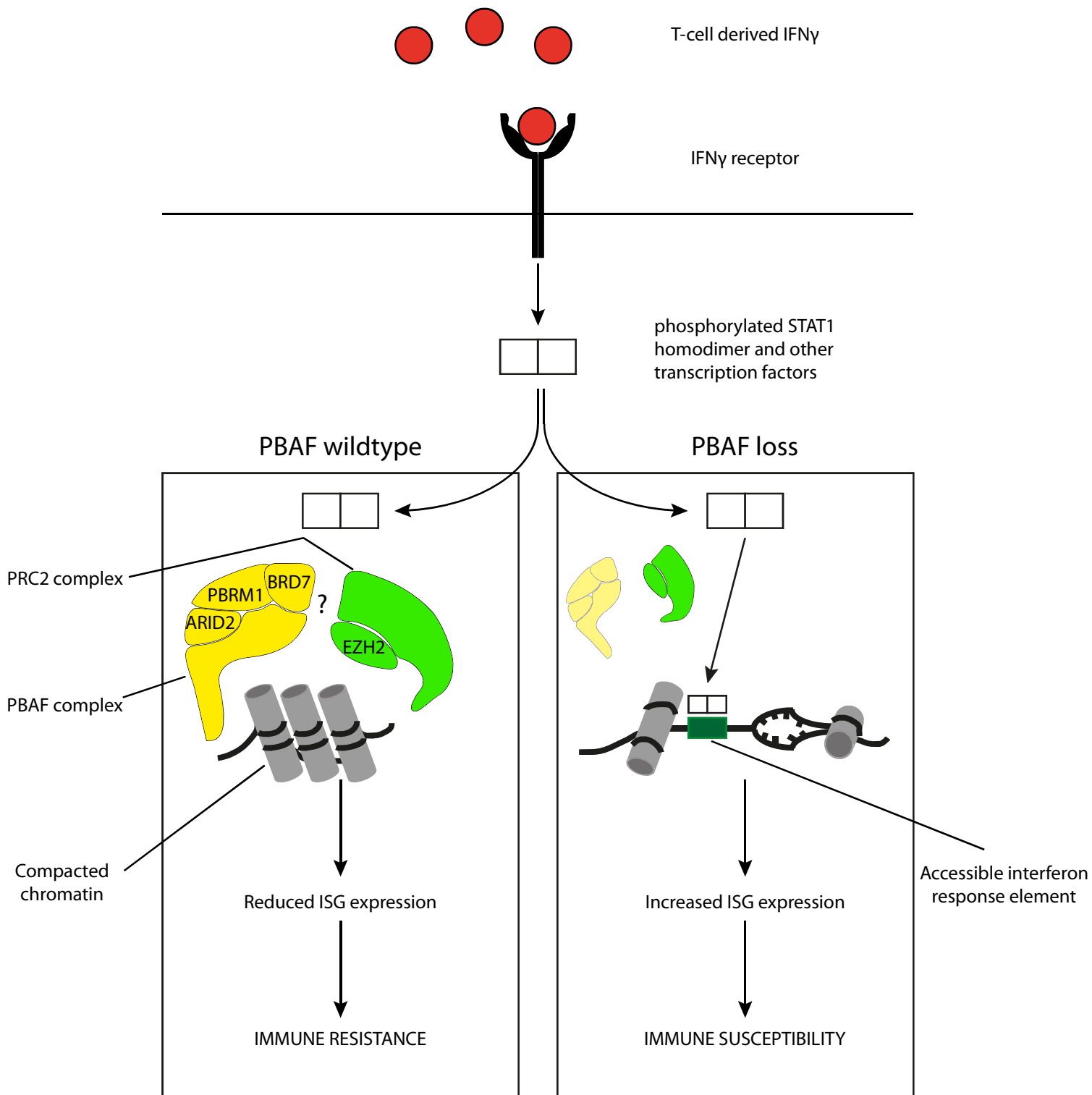
As PBAF loss-of-function mutation or decreased expression is seen in multiple cancer types other than ccRCC, the finding that PBAF deficiency may enhance tumor cell susceptibility to immune control could have wider translational importance. In tumor types with greater CPI sensitivity, such as ccRCC, PBAF deficiency may serve as a biomarker of treatment response and so aid patient selection. Together with the recent demonstration that EZH2 inhibition enhances CPI efficacy in mouse models of melanoma (15), the findings of Pan *et al.* and Miao *et al.* pave the way for clinical trials combining small-molecule inhibitors of chromatin remodeling pathways and CPIs as a new synergistic

combination. Broadening the question beyond tumor cell–intrinsic mechanisms that contribute to CPI sensitivity in the context of altered chromatin remodeling, one important question for future work is whether there are reciprocal effects of tumor cell chromatin regulation on T cell function and the landscape of immune checkpoint expression that may enable further refinement of therapeutic strategies.

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Figure title: Mutation of genes forming the chromatin remodeling complex PBAF sensitizes cancer cells to interferon-mediated immune destruction.  
Caption: Human ccRCC tumors from checkpoint immunotherapy responders were enriched for PBRM1 loss-of-function mutations. In a mouse CRISPR screen, deletion of PBAF complex defining genes *PBRM1*, *ARID2*, or *BRD7* resulted in increased ISG expression and sensitized tumors to checkpoint immunotherapy. PBAF deficiency enhanced accessibility of interferon response motifs. A possible mechanism for this effect may involve PBAF cooperation with the PRC2 complex protein EZH2 to reduce chromatin accessibility.



Mutation of genes forming the chromatin remodelling complex PBAF sensitises cancer cells to interferon mediated immune destruction. Human ccRCC tumours from checkpoint immuno-therapy responders were enriched for PBRM1 loss of function mutations. In a mouse CRISPR screen, knock-out of PBAF complex defining genes PBRM1, ARID2 or BRD7 resulted in upregulated ISG expression and sensitised tumours to checkpoint immu-notherapy. PBAF deficiency en-hanced accessibility of interferon re-sponse motifs. A possible mechanism for this effect may involve PBAF co-operation with the PRC2 complex protein EZH2 to reduce chromatin accessibility.