Activation of NF-B and p300/CBP potentiates cancer chemoimmunotherapy through induction of MHC-I antigen presentation

Zhou, Yixuan; Bastian, Ingmar Niels; Long, Mark D.; Dow, Michelle; Li, Weihua; Ngu, Rachael Katie

Published in:
Proceedings of the National Academy of Sciences

DOI:
10.1073/pnas.2025840118

Publication date:
2021

Document Version
Publisher's PDF, also known as Version of record

Link to publication in Discovery Research Portal

Citation for published version (APA):
Zhou, Y., Bastian, I. N., Long, M. D., Dow, M., Li, W., Ngu, R. K., Antonucci, L., Huang, J. Y., Phung, Q. T., Zhao, X., Banerjee, S., Lin, X-J., Wang, H., Dang, B., Choi, S., Karin, D., Su, H., Ellisman, M. H., Jamieson, C., ... Shalapour, S. (2021). Activation of NF-B and p300/CBP potentiates cancer chemoimmunotherapy through induction of MHC-I antigen presentation. Proceedings of the National Academy of Sciences, 118(8), [e2025840118]. https://doi.org/10.1073/pnas.2025840118
Activation of NF-κB and p300/CBP potentiates cancer chemoinmunotherapy through induction of MHC-I antigen presentation

Yixuan Zhoua,b,1, Ingmar Niels Bastiana,d,1, Mark D. Longb,1, Michelle Dowd,1,2, Weihua Li,1,3,1, Tao Liu,1,3,1, Rachael Katie Ngu,4, Laura Antonucci,a,e,1, Jian Yu Huang,4,5,6, Qui T. Phung,4,7, Xi-he Zhao,4,8,9, Sourav Banerjee,a,e,1, Xue-Jia Lin,4,5,6, Hongxia Wang,a,b, Brian Dang,4,8, Sylvia Choi,1,8, Daniel Karina,9, Hua Su,1,2,1, Mark H. Ellisman,1, Christina Jamiesona,1, Marcus Bosenga,1,2,3,4, Zhang Cheng,1,2,1, Johannes Haybaec,1,2, Lukas Kenner,1,3, Kathleen M. Fisch,1, Richard Bourgona, Genevive Hernandez,4, Jennie R. Lilla,1, Song Liu,1, Hannah Carter,1,2, Ira Mellauna,1,2, Michael Karina,2,3, and Shabnam Shalapoura,1,2,1

1Department of Pharmacochemistry, School of Medicine, University of California San Diego, CA 92093; 2Department of Biostatistics and Bioinformatics, Roswell Park Comprehensive Cancer Center, Buffalo, NY 14263; 3Division of Medical Genetics, Health Sciences, Department of Biomedical Informatics, University of California San Diego, La Jolla, CA 92093; 4Department of Medicine, University of California San Diego, La Jolla, CA 92093; 5Laboratory of Gene Regulation and Signal Transduction, Department of Pharmacochemistry, School of Medicine, University of California San Diego, La Jolla, CA 92093; 6Department of Microchemistry, Proteomics, and Lipidomics, Genentech, Inc., South San Francisco, CA 94080; 7Oncology Department, China Medical University Shengjing Hospital, 110004 Shenyang City, China; 8Department of Cellular Medicine, Jacqui Wood Cancer Centre, University of Dundee, Dundee DD1 9YX, United Kingdom; 9Biomedical Translational Research Institute and the First Affiliated Hospital, Jinan University, 510632 Guangzhou, Guangdong, China; 10State Key Laboratory of Proteomics, Institute of Basic Medical Sciences, National Center of Biomedical Analysis, 100850 Beijing, China; 11National Center for Microscopy and Imaging Research, Center for Research in Biological Systems, University of California San Diego, La Jolla, CA 92039; 12Department of Urology, Moores Cancer Center, University of California San Diego, La Jolla, CA 92093; 13Department of Immunobiology, Yale School of Medicine, New Haven, CT 06510; 14Department of Dermatology, Yale School of Medicine, New Haven, CT 06510; 15Center for Epigenomics, Department of Cellular and Molecular Medicine, School of Medicine, University of California San Diego, La Jolla, CA 92039; 16Institute of Pathology, Medical University of Graz, A-8036 Graz, Austria; 17Department of Pathology, Neuropathology and Molecular Pathology, Medical University of Innsbruck, A-6020 Innsbruck, Austria; 18Department of Pathology, Christian Doppler Laboratory, Medical University of Vienna, 1090 Vienna, Austria; 19Unit of Pathology of Laboratory Animals, University of Veterinary Medicine Vienna, 1210 Vienna, Austria; 20Center for Computational Biology and Bioinformatics, Department of Medicine, University of California San Diego, La Jolla, CA 92039; 21Department of Cancer Immunology, Genentech, Inc., South San Francisco, CA 94080; and 22Department of Cancer Biology, University of Texas MD Anderson Cancer Center, Houston, TX 77054

Many cancers evade immune rejection by suppressing major histocompatibility class I (MHC-I) antigen presentation (AgPP). Such cancers do not respond to immune checkpoint inhibitor therapies (ICT) such as PD-1/PD-L1 [PD-(L)1] blockade. Certain chemotherapeutic drugs augment tumor control by PD-(L)1 inhibitors through potentiation of T-cell priming but whether and how chemotherapy enhances MHC-I-dependent cancer cell recognition by cytotoxic T cells (CTLs) is not entirely clear. We now show that the lysine acetyl transferases p300/CREB binding protein (CBP) control MHC-I AgPP expression and neoantigen amounts in human cancers. Moreover, we found that two distinct DNA damaging drugs, the platinoi oxaliplatin and the topoisomerase inhibitor mitomycin, strongly up-regulate MHC-I AgPP in a manner dependent on activation of nuclear factor kappa B (NF-κB), p300/CBP, and other transcription factors, but independently of autocrine IFNγ signaling. Accordingly, NF-κB and p300 ablations prevent chemotherapy-induced MHC-I AgPP and abrogate rejection of low MHC-I-expressing tumors by reinvigorating CD8+ CTLs. Drugs like oxaliplatin and mitomycin can be used to overcome resistance to PD-(L)1 inhibitors in tumors that had “epigenetically down-regulated,” but had not permanently lost MHC-I AgPP activity.

Significance

T cells recognize their targets via their T-cell receptors (TCRs), which in the case of CD8+ T cells bind to MHC-I-antigen complexes on the surface of target cells. Many cancer cells evade immune recognition and killing by down-regulating MHC-I AgPP. Here, we show how the histone acetyl transferases p300/CBP together with NF-κB epigenetically regulate expression of MHC-I molecules, immunoproteasome subunits, and peptide transporter to enable proper MHC-I antigen presentation. Notably, this pathway is frequently disrupted in human cancers. We now show that certain chemotherapeutics can augment MHC-I antigen presentation via NF-κB and p300/CBP activation, thereby enhancing cancer cell recognition and killing by effector CD8+ CTLs.

Author contributions: M.K. and S.S. designed research; Y.Z., I.N.B., M.D.L., M.D., W.L., T.L., R.K.N., L.A., J.Y.H., Q.T.P., X.-H.Z., S.B., X.-J.L., H.W., B.D., S.C., H.S., Z.C., and S.S. performed research; C.J., M.B., J.H., L.K., and I.M. contributed new reagents/analytic tools; Y.Z., I.N.B., M.D.L., M.D., W.L., T.L., R.K.N., L.A., J.Y.H., Q.T.P., X.-H.Z., S.B., X.-J.L., H.W., B.D., S.C., H.S., M.H.E., Z.C., K.M.F., R.B., G.H., J.R.L., S.L., H.C., I.M., M.K., and S.S. analyzed data; and M.K. and S.S. wrote the paper.

Reviewers: J.M.B., Weill Cornell Medicine; and W.Z., University of Michigan.

Competing interest statement: I.M., J.R.L., R.B., Q.T.P. and G.H. are employees of Genentech.

This open access article is distributed under Creative Commons Attribution License 4.0 (CC BY).

1Y.Z., I.N.B., M.D.L., M.D., and W.L. contributed equally to this work.

To whom correspondence may be addressed. Email: m.karin@ucsd.edu or sshalapour@mdanderson.org.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2025840118/-/DCSupplemental.

Published February 18, 2021.

PNAS 2021 Vol. 118 No. 8 e2025840118
https://doi.org/10.1073/pnas.2025840118 | 1 of 12
(ICD) by radiotherapy and chemotherapy (14). By enhancing the release of damage associated molecular patterns (DAMPs) and other molecules, ICD stimulates tumor antigen uptake by antigen-presenting cells (APCs) that prime T cells against tumor antigens, as demonstrated by vaccination experiments (15). Primed T cells may accumulate in the tumor and lead to immune rejection as long as they can recognize and kill their targets (16). Such strategies are ineffective in cancers with low MHC-I or HLA-A/B/C expression (11, 16–19).

PCAs is a typical ICT-refractory cancer, presumably due to low expression of HLA-A/B/C molecules that together with β2 microglobulin form MHC-I heterodimers, which present tumor antigens to CD8+ CTL (20, 21). Using mouse models of PCa we found that the platin-based DNA-crosslinker oxaliplatin (Oxali) potentiates immune rejection of autochthonous or engrafted tumors after genetic or pharmacological depletion of PD-L1–expressing immunosuppressive IgA+ plasmocytes, which cause CTL exhaustion (22). Low-dose Oxali also enhances mouse PCa regression in response to anti–PD-L1 treatment (22). Similar results were obtained with low-dose Oxali or photodynamic therapy in other cancer models (23, 24) but the underlying mechanisms have not been explored. As Oxali is known to induce IC and T-cell priming, we investigated its ability to potentiate the immune rejection of IgA+ plasmocyte-depleted or anti–PD-L1–treated low MHC-I prostate tumors also entails effects on the recognition and killing step of the cancer-immunity cycle, which depends on CTL–MHC-I interactions (16, 25). Here we show that Oxali and the structurally unrelated toposomerase II inhibitor mitoxantrone (Mito) transcriptionally up-regulate expression of MHC-I molecules and their cognate antigen presentation and processing machinery (AgPPM). This response, which takes place in human and mouse cancers, depends on activation of nuclear factor kappa B (NF-κB) and nuclear translocation of the closely related histone (and lysine) acetyltransferases p300 and CREB binding protein (CBP). Whereas p300 ablation abrogated MHC-I AgPP induction and the synergy between low-dose Oxali and PD-(L)1 blockade, it had no effect on induction of antitumor immunity by Oxali-killed PCa cells used as an immunogen.

Results
Oxaliplatin and Mitoxantrone Induce MHC-I AgPPM Genes. To determine the effect of Oxali and related drugs on gene expression in PCa cell lines used in our previous study (22), Myc-CaP cells were treated with different drugs at doses that induce no more than 10% cell death, and vital cells (SI Appendix, Fig. S1 A and B) were analyzed by whole genome RNA sequencing (RNA-seq) and assay for transposable-accessible chromatin (ATAC-seq) (26). Since CTL reactivation via anti–PD-L1 induces IFNγ production (27, 28), we also examined the effect of IFNγ alone or together with chemotherapy. Low-dose chemotherapeutic, in particular Oxali, induced marked changes in gene expression and chromatin accessibility depicted as differentially expressed genes (DEGs) and differentially accessible DNA regions (DARS) (Fig. 1A and Appendix, Fig. S1 C–F). The platin-induced changes were usually augmented by IFNγ, although the effects of Oxali were broader than that of IFNγ, which mainly enhanced gene expression magnitude rather than breadth. Some of the Oxali or IFNγ-induced gene sets were common to both agents (Fig. 1A). Pathway enrichment analysis (Fig. 1B) identified the most significantly enriched pathways, activated by Oxali (red, e.g., epithelial-mesenchymal transition, TP53), IFNγ (blue, e.g., Myc), or Oxali + IFNγ together (purple, e.g., IFN type I and II, AgPPM). Notably, while either Oxali or IFNγ significantly enriched genes involved in MHC-I AgPP and IFNγ signaling, these effects were strongly enhanced when Oxali and IFNγ were combined (Fig. 1B and SI Appendix, Fig. S2 A–C). However, Oxali did not induce IFNγ expression, indicating that its ability to induce MHC-I AgPPM components was not due to autocrine IFNγ signaling.

Pathway enrichment analysis of DEGs that were responsive to Oxali plus IFNγ revealed strong induction of genes related to type I and II IFN signaling and MHC-I AgPPP components, involved in protein folding, MHC-I complex assembly, and peptide loading, as well as genes involved in the endoplasmic reticulum (ER)–phagosome pathway and antiviral responses (Fig. 1C and SI Appendix, Fig. S2D). Most of these genes were also induced by Oxali alone. To understand how these genes were induced we examined the ATAC-seq patterns of a gene cluster on mouse chromosome 17 harboring the Psnmb, Tap1, Pomb8, and Tap2 genes, coding for immunoproteasome components and peptide transporters (SI Appendix, Figs. S1 E and F and S3A). Low-dose Oxali, and to a lesser extent cisplatin (Cispl), increased transcription factor (TF) accessibility at several sites within this locus (SI Appendix, Fig. S3A). Surprisingly, IFNγ alone had little effect, if any, on chromatin structure (SI Appendix, Fig. SIF). qRT-PCR analysis further confirmed induction of AgPPM genes by Oxali and Cispl and to a lesser extent by Mito, alone or together with IFNγ (SI Appendix, Fig. S3 B–D). A similar response pattern was displayed by the Nlrc5 gene coding for NLRCS/CITA, the master activator of the MHC-I AgPPP (Fig. 2A). ATAC-seq revealed increased Nlrc5 chromatin accessibility after low-dose Oxali or Cispl, but hardly any change after IFNγ treatment (Fig. 2B). Mito, Cispl, and Oxali, but not IFNγ, induced Plsmb8 mRNA, but had little effect on chromatin accessibility of its gene (Fig. 2 C and D). Of note, the chromosome 17 region opened up by Oxali contains binding motifs recognized by BORIS and CTSCF (SI Appendix, Fig. S3A), general TF responsible for chromatin opening (29). Low-dose Oxali also increased β2 microglobulin (β2M), and all tested chemotherapeutics induced surface and mRNA expression of H-2Kq, the predominant MHC-I molecule in Myc-CaP cells (Fig. 2 E and F and SI Appendix, Fig. S3D). Low-dose Oxali increased immunoproteasome activity measured with an LMP7/PSMB8-specific substrate, Ac-ANW-AMC, an effect that was potentiated by IFNγ (Fig. 2G).

Putative Transcriptional Regulators of MHC-I AgPPM Induction. We searched for signaling pathways and TF-mediating MHC-I AgPPP and IFNγR2 induction by low-dose Oxali, RNA-seq and pathway enrichment analysis suggested involvement of IRF, STAT, NF-κB, MYC family members, and androgen receptor (AR) (Fig. 2 H–K and SI Appendix, Fig. S3 E and F). Whereas the IRF, STAT, and NF-κB pathways were up-regulated by Oxali and potentiated by IFNγ, the MYC and to a lesser extent the AR pathway, both of which participate in PCa tumor genesis (30–32), were down-regulated after Oxali + IFNγ treatment. Among IRF family members, IRF1, 7, and 9 were stimulated by Oxali and IFNγ, IRF2 was induced by Oxali, and IRF8 mainly responded to IFNγ (SI Appendix, Fig. S3F). Similarly, STAT1 and 2 were stimulated by Oxali, whereas IFNγ induced STAT1 and 3. JUN, ATF3, UBA7, CREB3, NFE2L1, and SOCS1 were induced by low-dose Oxali, along with NF-κB1 (p105) and NF-κB2 (p100) (SI Appendix, Fig. S3F). ATAC-seq confirmed that Oxali, but not IFNγ, enhanced chromatin accessibility of the Nfkb1 locus (Fig. 2H).

We employed two additional analytic approaches to identify master TF-mediating treatment-induced expression changes [LISA (33)] and TF binding enrichment within regions of differential chromatin accessibility [GIGGLE (34)]. These analyses predicted the master regulators (MRs) most likely to influence the DEGs (Fig. 2J) and DARS (Fig. 2F) by leveraging the complete set of TF binding datasets available from the CistromeDB collection. The results further highlighted treatment-related directional TF associations (up/down-regulated DEGs, open/closed DARS); Oxali: ESR1, NR3C1, and JUN; IFNγ: MYC, STAT1, ATF4, and FOS; Oxali + IFNγ: STAT1, YAP1, ESR1, IRF1, RELA, and IRF8 (Fig. 2 I and J). DEG and DAR
integration revealed 200 common TFs, including IRFs and STATs (Fig. 2K). Notably, Oxali treatment elicited marked changes in histone methylation- and acetylation-related gene signatures (Fig. 2L) in agreement with the ATAC-seq data (SI Appendix, Fig. S1E and F). Subset analyses focusing on histone modifying factors implicated the involvement of the histone acetyltransferases (HATs) p300 and CBP and several histone deacetylases (HDACs) (SI Appendix, Fig. S4A–G).

Chemotherapy Stimulates HAT Nuclear Localization and Activity. Chromatin structure opening, as revealed by ATAC-seq analysis, depends on histone acetylation (35). Importantly, Oxali treatment of Myc-CaP cells, increased HAT enzymatic activity within 3 h and its effect was comparable to that of a HDAC inhibitor (HDACi) (Fig. 3A and B). Oxali and Mito also increased total p300, acetylated CBP/p300, and K310-acetylated RELA/p65 nuclear amounts (Fig. 3C). IFNγ also increased nuclear p300, but its effect was considerably weaker than that of

Fig. 1. Chemotherapy induces MHC-I AgPPM genes. (A) Heatmap showing all DEGs identified in bulk RNA-seq of Myc-CaP cells treated with IFNγ (0.2 or 2 ng/mL), Oxali (2 μM), or both (combo) (Left). Venn diagram shows overlapping DEGs between IFNγ (2 ng/mL), Oxali, and combo treatment groups relative to Ctrl (Middle) and heatmap shows all DARs identified in bulk ATAC-seq of Myc-CaP cells treated as indicated relative to control (Right). (B) Gene set enrichment analysis (GSEA) was applied to expression profiles specific to each treatment group relative to Ctrl. Top 30 significantly enriched pathways for each respective comparison are shown. Some pathways were considered both IFNγ and Oxali driven (purple), while others were specific to either IFNγ (blue) or Oxali (red). (C) Functional enrichment was applied to genes classified with additive response to combination therapy. The top 20 enriched REACTOME pathways are shown.
Fig. 2. Transcriptional regulators of Oxali-induced MHC-I AgPPM genes. (A) RNAs from Myc-CaP cells incubated as indicated with IFNγ, Mito, Oxali, carboplatin (Carbo), or Cispl for 48 h were analyzed by qRT-PCR using Nlrc5 primers. (B and C) Candidate genomic loci for Nlrc5 (B) and Ifngr2 (C), showing library-size normalized read pair pileup profiles determined by ATAC-seq across samples. Expression of respective genes determined by RNA-seq is also shown. (D) RNAs from Myc-CaP cells incubated as indicated were analyzed by qRT-PCR using Ifngr2 primers. (E) Myc-CaP cells incubated with Oxali for 12 h were stained with β2M antibody (green) and phalloidin (red) and counterstained with DAPI. (Scale bar: 20 μm.) (F) Myc-CaP cells treated as indicated were analyzed for surface MHC-I (H-2Kq) expression by flow cytometry. (G) Myc-CaP cells were incubated as indicated and lysed. LMP7 (PSMB8) immunoproteasome activity was measured using a fluorogenic LMP7-specific substrate peptide Ac-ANW-AMC. (H) Candidate genomic locus for Nfkb1 showing read density profiles determined by ATAC-seq across samples. (I) LISA was applied to DEGs identified in comparisons of IFNγ, Oxali, and both (combo)-treated cells relative to control, as well as to genes classified with additive response (top 500 up-regulated, down-regulated DEGs). The top 20 enriched regulators of up-regulated (red) and down-regulated (blue) DEGs are noted. (J) GIGGLE applied to DARs identified in comparisons of IFNγ-, Oxali-, and combo-treated cells relative to control, as well as to regions classified with additive response. The top 20 enriched regulators of opened (red) and closed (blue) DARs are noted. (K) Two hundred common genes were identified by comparing TFs found by DEG and DAR analysis. (L) Gene set enrichment analysis (GSEA) was applied to expression profiles determined in Oxali-treated cells relative to control. The literature-curated known regulatory elements from ORegAnno database are shown. Candidate enrichment plots for representative pathways related to histone methylation (Top) and acetylation (Bottom) are shown. Two-sided t test (means ± SEM), and Mann–Whitney test (median) were used to determine significance between two groups. One-way ANOVA analysis (A, D, F, G) and multiple comparison confirmed the results. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Specific n values are shown in A, D, F, and G, each experiment includes at least three biological replicates.
Oxali (Fig. 3 C and D). Both Oxali and HDACi induced p300 nuclear translocation in murine PCa cells (Fig. 3D) and human PCa organoids (SI Appendix, Fig. S5A). Chromatin immunoprecipitation (ChIP) experiments showed that Oxali induced p300 and RELA/p65 recruitment to the Nlrc5 and Ifngr2 promoters and p300 recruitment to the Tap1 and Psmrb8/9 promoters (Fig. 3 E and F). These promoter regions also exhibited increased H3K14 and K27 acetylation after Oxali treatment (Fig. 3G). Oxali-induced H3K14 acetylation at nuclear foci, similar to those revealed by p300 antibody staining, was also observed by immunofluorescence (IF) analysis (SI Appendix, Fig. S5B). Increased RELA/p65 K310 acetylation, which was attenuated after treatment by p300/CBP inhibitors, was confirmed by IB and IF analyses (SI Appendix, Fig. S5C and D). Using HA- or Myc-tagged p300 and Flag-tagged Stat1 expression vectors followed by immunoprecipitation (IP), we confirmed binding of p300 to endogenous RELA/p65 and transfected STAT1 (SI Appendix, Fig. S5E), an interaction that stimulates p300 acetyltransferase activity (36). To investigate the basis for p300 nuclear translocation, we examined induction of HLA-B–associated transcript 3 gene product, BAT3, which controls intracellular p300 distribution (37). IF analysis confirmed Oxali-induced nuclear translocation of both p300 and BAT3 (SI Appendix, Fig. S5F).

**Fig. 3.** Oxaliplatin and mitoxantrone stimulate HAT activity and nuclear localization. (A and B) Myc-CaP cells incubated with Oxali (2 or 4 μM) or the HDACi panobinostat (LBH589; 20 nM) for the indicated times were lysed and analyzed for HAT activity using H3 as a substrate. (C) Nuclear extracts of Myc-CaP cells treated with Oxali, Mito, and/or IFN-γ were IB analyzed for p300, acetylated-CBP/p300, acetylated-RELA/p65 (lysine K310), and lamin B1 (loading control). (D) Myc-CaP cells treated as indicated for 12 h were stained with anti-p300 (green) and phalloidin (red; actin cables). Nuclei were counterstained with DAPI (blue). (Scale bar: 20 μm.) (E–G) Untreated and Oxali-treated Myc-CaP cells were subjected to ChIP analysis with control IgG and antibodies to p65/RelA, p300 as indicated (E and F) or acetylated H3 (lysine K9, K14, and K27) (G). Precipitation of the indicated promoter regions was determined by PCR. Two-sided t test (means ± SEM), and Mann–Whitney test (median) were used to determine significance. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Specific n values are shown in A and E; each experiment includes at least three biological replicates.

Zhou et al. PNAS | 5 of 12
Activation of NF-κB and p300/CBP potentiates cancer chemoimmunotherapy through induction of MHC-I antigen presentation

https://doi.org/10.1073/pnas.2025840118

IMMUNOLOGY AND INFLAMMATION

Credit: See end of article.
Consequently, both deficiencies hampered Oxali- and Mito-induced H-2Kq mRNA and surface expression (Fig. 4 and SI Appendix, Figs. S6 F and G). A p300/CBP inhibitor also attenuated H-2Kq protein, and Psmb9 and Tap1 mRNA inductions (SI Appendix, Figs. S6 H and I). Conversely, treatment of Myc-CaP cells with nonlethal doses of the HDACi panobinostat (LBH589) induced Nlr5, Psmb9, and Tap1 mRNAs and surface H-2Kq (SI Appendix, Fig. S6 J–L).

To gather information about p300 and CBP in human cancer, we examined The Cancer Genome Atlas (TCGA) dataset and found significant correlations between EP300 or CBP mRNAs and genes identified by our integrative RNA-seq and ATAC-seq analyses, including RELA, STAT1, NFKB1, IFNGR2, and NLRC5 (Fig. 5 A and B and SI Appendix, Fig. S7A). Similar correlations were found between histone modifiers and genes involved in MHC-I AgPP and T-cell inflammation, particularly in human liver cancer (SI Appendix, Fig. S7 B and C). Cancers with EP300/CBP loss of function (LOF; deletion and/or copy number variants [CNV] loss) showed lower ERAP1 and IFNGR1 or HLA-A expression (Fig. 5B). Curiously, EP300 and CBP were described both as oncogenes and oncosuppressors (39, 40). Phenotypes associated with heterozygous alterations were described in B cell lymphoma and Rubinstein-Taybi syndrome 1 (41, 42), suggesting association with heterozygous alterations were described in B cell lymphoma and Rubinstein-Taybi syndrome 1 (41, 42), suggesting correspondence of both organelles (Fig. 6 E). Electron microscopy (EM) suggested that Oxali-treated cells underwent nucleolar/mitochondrial fragmentation confirmed that most of the stressed cells remained viable. Oxali-induced nucleolar/ribosomal stress (45, 46), which was confirmed by mass spectrometry (MS) and RNA-seq analyses (SI Appendix, Fig. S9 B–E), can account for NF-κB activation (47). To determine NF-κB’s role in the response to Oxali, we generated RELA/p65-deficient cell lines (SI Appendix, Fig. S10 A and B). Consistent with the ChIP experiments shown

**EP300 and CBP copy numbers correlated with improved LIHC patient survival (Fig. 5C). We also analyzed CNVs of genes involved in MHC-I AgPPM and IFNγ signaling (Fig. 5 D and E and SI Appendix, Fig. S8 and Table S1). We found that LIHC and PRAD patients with gains in MHC-I AgPPM genes showed more frequent EP300/CBP LOF (Fig. 5 D and E and SI Appendix, Table S1), suggesting a compensatory mechanism that allows cancers with elevated MHC-I AgPPM to evade immune recognition. We also analyzed the number and the fraction of neoantigens in different cancers (SI Appendix, Fig. S8 B and C). Remarkably, cancers with EP300/CBP LOF showed higher neoantigen amount (Fig. 5F and SI Appendix, Fig. S8D), supporting the notion that tumor immunoediting shapes the neoantigen landscape (43, 44) and that EP300/CBP may be part of this process.

**NF-κB Signaling and MHC-I AgPPM Induction.** Electron microscopy (EM) suggested that Oxali-treated cells underwent nucleolar/ribosomal and mitochondrial stress indicated by the condensed appearance of both organelles (Fig. 6 A and B and SI Appendix, Fig. S9A). However, the absence of nuclear or mitochondrial fragmentation confirmed that most of the stressed cells remained viable. Oxali-induced nucleolar/ribosomal stress (45, 46), which was confirmed by mass spectrometry (MS) and RNA-seq analyses (SI Appendix, Fig. S9 B–E), can account for NF-κB activation (47). To determine NF-κB’s role in the response to Oxali, we generated RELA/p65-deficient cell lines (SI Appendix, Fig. S10 A and B). Consistent with the ChIP experiments shown

Fig. 4. p300 and CBP control Oxali-induced MHC-I AgPPM genes. (A) Parental (shRNA-Ctrl) and p300 or CBP silenced Myc-CaP cells were incubated with Oxali for 48 h. Nuclear extracts were IB analyzed for p300, CBP, p65/RELA, and HDAC1 (loading control). (B–G) RNA expression in above cells was analyzed by qRT-PCR with the indicated primers. (H) Parental and gene-edited Myc-CaP cells were incubated with Oxali and analyzed for surface MHC-I (H-2Kq) expression by flow cytometry. Two-sided t test (means ± SEM), and Mann-Whitney test (median) were used to determine significance unless indicated otherwise. *(P < 0.05); **P < 0.01; ***P < 0.001; NS, not significant. Specific n values are shown in B–H. Each experiment includes at least three biological replicates.
ANOVA analysis and multiple comparison confirmed the results. 

**cutaneous melanoma (SKCM; TCGA mRNA-seq reads. Two-sided termine significance. (**

*...*

of < 0.5 were considered binding and strong binding, respectively. 

Expression of neoantigens was determined from TCGA mRNA-seq reads. Two-sided t test (means ± SEM), and Mann-Whitney test (median) were used to determine significance unless indicated otherwise. One-way ANOVA analysis and multiple comparison confirmed the results. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Specific n values are shown in A–F. Each experiment includes at least three biological replicates.

above (Fig. 3E), RELA/p65 was needed for full induction of Iftn1, Tap1, Psmab9, Nlr3, and Bat3 mRNAs by low-dose Oxali (Fig. 6 C–E and SI Appendix, Fig. S10 C and D). RELA/p65 abla-tion strongly inhibited surface H-2Kq induction by Oxali but barely affected the response to IFNγ (Fig. 6F). BAT3 and p30 nuclear translocation was also attenuated in RELA-deficient cells (Fig. 6G and SI Appendix, Fig. S10E). Treatment of Myc-CaP cells with IKKβ inhibitors also reduced H-2Kq surface expression (SI Appendix, Fig. S10F). We also generated Irf-1-, Sting/cGAS-, Stat1-, IftnγR2-, and Vdac1-deficient Myc-CaP cells (Fig. 6H and SI Appendix, Fig. S11 A–E). Vdac1 (voltage-dependent anion channel 1) was recently shown to be required for the cytoplasmic release of mitochondrial (mt) DNA (48), which is considerably elevated in Oxali-stressed cells (SI Appendix, Fig. S11 E and F). Notably, Vdac1 ablation strongly reduced Rela, p300, and Iftnγ mRNA induction by Oxali (SI Appendix, Fig. S11G). Ablation of Vdac1 and Iftnγ, but not Stat1 or IftnγR2, abrogated Oxali-induced expression of surface H-2Kq and Mhc-I Agppm genes (Fig. 6H and SI Appendix, Fig. S11 H–K). Ablation of cGAS led to a small decrease in H-2Kq expression and no effect on induction of most Agppm genes (Fig. 6H and SI Appendix, Fig. S11 H–K). Not surprisingly, Stat1, Irf-1, and IftnγR2 as well as Vdac1 and cGAS were required for H-2Kq surface expression in Myc-CaP cells treated with IFNγ alone or IFNγ + Oxali (SI Appendix, Fig. S11L). Oxali treatment also led to modest induction of PD-L1, a response that was enhanced by exogenous IFNγ and was Iftnγ dependent (SI Appendix, Fig. S11L).
Appendix, Fig. S11M), which has previously been shown to contribute to efficacy of ICIT (49). PD-L1 induction was not affected by TAPI ablation, which completely prevented H-2Kq surface expression.

Chemotherapy-Induced Functional Antigen Presentation. To confirm that Oxali stimulates neoantigen presentation, we used MS to determine the peptidomes of H-2Kb and H-2Db molecules.

Fig. 6. Role of NF-κB signaling in Oxali-induced MHC-I AgPPM expression. (A and B) Myc-CaP cells treated as indicated were fixed and examined by electron microscopy. Magnification bars are indicated in each image. White arrows indicate nucleolar stress. Ten representative images from each treatment group were analyzed and mitochondria/cytoplasm ratios were determined (B, Right). (C–E) Parental (shRNA-Ctrl) or Relap65-silenced Myc-CaP cells were incubated with Oxali as indicated. RNAs were analyzed by qRT-PCR with the indicated primers. The results were confirmed using three different Relap65 shRNAs. (F) Myc-CaP cells described in C were treated as indicated and analyzed for surface MHC-I (H-2Kq) expression. (G) Parental or Relap65-silenced Myc-CaP cells were treated as indicated and stained with mouse anti-p300 (green) and rabbit anti-BAT3 (red). Nuclei were counterstained with DAPI (blue) (n = 3). (Scale bar: 20 μm.) (H) Parental and gene edited (Ctrl, CRISPR-Cas9) or shRNA silenced Myc-CaP cells were treated as indicated and analyzed for surface MHC-I (H-2Kq) expression. (B, F, and H) Two-sided t test (means ± SEM), and Mann-Whitney test (median) were used to determine significance. One-way ANOVA and multiple comparisons were used to confirm significance. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Specific n values are shown in B–F. Each experiment includes at least three biological replicates.

Activation of NF-κB and p300/CBP potentiates cancer chemoimmunotherapy through induction of MHC-I antigen presentation.
isolated from MC-38 cells after treatments, as described previously (50). Treatment with IFNγ + Oxali induced higher amounts (based on area under the curve) of H-2Kb-bound peptides relative to Oxali or IFNγ alone (SI Appendix, Fig. S12A). Although IFNγ led to higher amounts of H-2Db-bound peptides than Oxali in this particular cell line, chosen for its high MHC-I expression, some peptides were more efficiently presented after IFNγ + Oxali treatment.

The T-cell activating ability of the Oxali-induced MHC-I bound peptides was confirmed using TRC2 PCa cells expressing high-, medium-, and low-affinity ovalbumin (Ova) variants. Oxali treatment stimulated H-2Kb presentation of the SIINFEKL epitope, especially in TRC2-N4 cells made to express the high-affinity (wild-type [WT]) variant (SI Appendix, Fig. S12B). When incubated with OT-I CD8+ T cells, whose T-cell receptor (TCR) is SIINFEKL specific, Oxali-treated TRC2-N4 cells were more readily killed by activated OT-I T cells (SI Appendix, Fig. S12 C and D). OT-I T cells enhanced presentation of the WT SIINFEKL epitope by TRC2-N4 cells in the absence of Oxali but had no effect on cells expressing the medium (TRC2-G4)- or low (TRC2-E1)-affinity variants. These results are consistent with a previous publication showing that only the high-affinity SIINFEKL epitope induces IFNγ secretion by OT-I cells (51), and further establish that the effect of Oxali is mechanistically distinct and that of IFNγ and dependent on neoantigen affinity and TCR activation.

We examined mouse and human cancer cell lines that differ in basal MHC-I expression. As described above, cells with high basal MHC-I such as MC-38 and B16 melanoma showed a weak response to platinoids alone but that response, including Nrc5 mRNA and surface MHC-I, was augmented by IFNγ (SI Appendix, Fig. S13 A and B). In other cancer cells, e.g., the mouse melanoma YUMM cell lines, we observed a considerable variation in the response (SI Appendix, Fig. S13C). Strong Oxali-induced MHC-I surface expression was detected in the human PC3 PCa cell line, PCSD1 cells, a three-dimensional (3D) organoid culture from a patient-derived xenograft (PDX) of bone metastatic PCa, certain primary melanoma cells, and Mia PaCa-2 cells, representing ICIT-refractory PDAC (SI Appendix, Fig. S13 D–G).

**Activation of p300/CBP and NF-kB Is Needed for Oxali + Anti-PD-L1 Synergy.** We sorted tumor-infiltrating CD8+ T cells (TC-CD8+) from subcutaneous (s.c.) Myc-CaP tumors, treated with either Oxali, anti–PD-L1, Oxali + anti–PD-L1 (combo), or left untreated (control [Ctrl]) and performed single-cell (sc)RNA-seq (Fig. 7A and SI Appendix, Fig. S14A). Several clusters of TC-CD8+ cells with distinguishable gene expression and cluster-specific pathway enrichment patterns were detected (Fig. 7B, and SI Appendix, Fig. S14 B–H). Notably, elevated GzmB, Gzam, Prf1, and Tnfl2(Tbet) mRNAs were detected in TC-CD8+ from combo-treated mice (SI Appendix, Fig. S14E). Only combo therapy was associated with a significantly higher T effector signature (Fig. 7C and SI Appendix, Fig. S14 F–H).

Next, we examined the involvement p300, CBP, IFNγR2, and NF-kB/RelA in Oxali-enhanced and CTL-mediated rejection of Myc-CaP tumors (Fig. 7A). The synergistic inhibition of tumor growth by Oxali + anti–PD-L1 was completely abrogated by p300 and CBP ablation in Myc-CaP tumors (Fig. 7D–G and SI Appendix, Fig. S15A). IFNγR2 ablation also abolished the response to Oxali + anti–PD-L1 (Fig. 7 G and H and SI Appendix, Fig. S15B). As found in vitro, low-dose Oxali induced expression of Ifng2, Tap1, Psmb9, and Nrc5 mRNA in Myc-CaP tumors (Fig. 7 I–K and SI Appendix, Fig. S15C). PD-L1 blockade did not affect Ifng2 mRNA expression, although it potentiated Tap1, Psmb9, and Nrc5 mRNA induction by Oxali, probably through IFNγ secretion by reinvigorated CTLs (Fig. 7 I–K and SI Appendix, Fig. S15C). Indeed, IFNγR2 ablation had little effect on the response to Oxali alone while abrogating the response to Oxali or Oxali + anti–PD-L1. Tap1, Psmb9, and Nrc5 induction by Oxali or Oxali + anti–PD-L1 was abrogated after p300 ablation (Fig. 7 I–K). IFNγR2 and p300 ablation also attenuated therapy-induced MHC-I (H-2Kq and H-2Dd) surface expression on CD4+ cancer cells (Fig. 7L and SI Appendix, Fig. S15 D and E), but had no effect on PD-L1 expression (SI Appendix, Fig. S15F). IFNγR2 and p300 ablations also had no effect on H-2Kq expression by tumor-infiltrating CD11c+ dendritic cells (SI Appendix, Fig. S15G). In accordance with scRNA-seq data, the Oxali + anti–PD-L1 combo increased the percentage and/or total numbers of tumor-infiltrating CD8+ and CD4+ T cells, CD107+IFNγ+ CTLs, IFNγ+ CD8+, and TNF+IFNγ+ CD8+ T cells, CD8+CD44+ Teff cells, and CD8+CD44+PD1+TIM-3+ T cells analyzed by flow cytometry (Fig. 7 M and N and SI Appendix, Fig. S16 C–H). Similar results were obtained for splenic CD8+ T cells (SI Appendix, Fig. S16 B and I–K). Of note, p300 or IFNγR2 ablation had little effect on tumor-infiltrating effector CD8+ T cells, whose numbers were similarly increased after Oxali + anti–PD-L1 treatment in p300- or IFNγR2-expressing and nonexpressing tumors (Fig. 7 M and N and SI Appendix, Fig. S16 C–H). By contrast, the Oxali + anti–PD-L1 combo decreased the fractions of each tumor occupied by CD45+ “cancer” cells, an effect that was most pronounced in WT tumors relative to p300 or IFNγR2 ablated tumors (Fig. 7O). NF-kB/RelA ablation also abolished the response to Oxali + anti–PD-L1 (Fig. 8A), consistent with its requirement for MHC-I and IFNγR2 induction (Fig. SB and SI Appendix, Fig. S17A). Oxali-induced up-regulation of MHC-I AgPPM genes in malignant cells is important for the final recognition and killing stage of the cancer-immunity cycle (25) but has no role in ICIT-induced CTL reinvigoration.

Of note, ICD-mediated T-cell priming proceeded normally in the absence of p300. FVB mice were immunized with Oxali-killed p300-proficient or -deficient Myc-CaP cells and challenged 1 wk later with vital p300-proficient or -deficient Myc-CaP cells (Fig. SC and SI Appendix, Fig. S17B). Myc-CaP cells grew significantly slower in FVB mice immersed with either p300-proficient or -deficient Myc-CaP cells compared to nonvaccinated mice, indicating that p300 has no effect on ICD-mediated T-cell priming.

**Discussion.**

ICIT-induced tumor rejection depends on activation of the cancer-immunity cycle, initiated by priming of tumor-directed T cells and terminated by killing of the targeted cancer cells by effector CTLs (16, 25). T-cell priming can be enhanced by certain chemotherapeutic drugs capable of inducing ICD (14) and ICIT (4, 27). Nonetheless, even highly effective T-cell priming and ICIT do not ensure successful CTL-mediated tumor killing, which requires MHC-I–mediated presentation of tumor-specific antigens (2, 14, 17, 52). Many cancers, especially PCa (SI Appendix, Fig. S7E) (53), evade immune elimination by down-regulating MHC-I molecules or essential AgPPM components (11). Here we show that two different chemotherapeutic drugs, Oxali and Mitoxantrone used at rather low concentrations, enhance CTL-mediated cancer cell recognition and killing through transcriptional induction of MHC-I AgPP (schematic summary, SI Appendix, Fig. S17C). Although induction of MHC-I antigen presentation by chemotherapy and radiotherapy was described (54–56), the underlying mechanisms were only partly explored and attributed to type I IFN signaling. However, recent reports showing that sustained type I IFN signaling contributes to anti–PD-(L)1 resistance (57, 58), cast doubt on the role played by type I IFN in chemotherapy- or radiotherapy-induced immune stimulation. Our results show that Oxali renders low MHC-I-expressing PCa cells responsive to anti–PD-(L)1 therapy through transcriptional activation of the MHC-I AgPPM by NF-kB and p300/CBP, but not via the IFN-
responsive TF STAT1. Ablation of p300 (or CBP) or NF-κB/RelA abolished the ability of low-dose Oxali to synergize with anti–PD-L1 and induce rejection of Myc-CaP tumors. Consistent with their direct involvement in transcriptional activation of MHC-I AgPPM genes, ablation of p300 or RelA abrogated Tap1, Psmb9, and Nlrc5 induction in Myc-CaP tumors, but had no effect on tumor infiltration by effector CD8+ cells. Tumor-infiltrating CTLs, however, were strongly increased after anti–PD-L1 + Oxali treatment as indicated by scRNA-seq and flow cytometry. In contrast, ablation of p300 had no effect on the ability of Oxali-killed...
down-regulation of NLRC5 has been observed in multiple cancer plex and NF-κ infiltration of CTLs.

Activation of NF-κB in activation of the MHC-I AgPP system. p300 plays a key activity via mitochondrial stress. Given the number of different signaling drugs. It is therefore understandable that their immunogenic infiltration of CTLs.

Neither Oxali nor Mito were developed as immunostimulatory drugs. It is therefore understandable that their immunogenic activity depends on multiple signaling pathways that are activated on induction of sublethal DNA damage and nuclear and mitochondrial stress. Given the number of different signaling pathways activated by Oxali or Mito, it is rather surprising that ablation of either p300/CFB or RelA results in almost complete inhibition of the drug-induced immunogenic response. These findings parallel the cardinal importance of p300/CBP and NF-κ-B signaling. However, the number of different cancer genes and PCa with gain of HLA, PSMB, and TAP genes, possibly due to chromosome 6p amplification (62), show higher frequency of CBP/EP300 loss, which may allow them to undergo immune evasion. Based on its loss in several types of cancer, EP300 was suggested to behave as a tumor suppressor gene (63, 64). We suggest that CBP/EP300 loss promotes tumor growth by enabling immune evasion. One way to restore recognition of tumors with monoallelic EP300/CBP loss is treatment with low-dose Oxali or Mito or more potent and specific EP300/CBP activators.

Myc-CaP cells to prime antitumor immunity, as indicated by vaccination experiments.

Oxali treatment triggers nucleolar/ribosomal stress (45, 46), possibly through its preferential interaction with rDNA or inhibition of rRNA synthesis, which represents almost half of the human genome (59). By virtue of its highly repetitive nature, rRNA integrity and expression are also sensitive to loss of topoisomerase II activity (60), a sequelae of Mito treatment. EM analysis of Oxali-treated Myc-CaP cells confirmed altered nucleolar morphology, consistent with nucleolar stress, which can trigger NF-kB activation (47). By inducing Bat3 transcription, RelA/NF-kB supports p300/CBP nuclear translocation, further increasing its own activity and stimulating histone acetylation. Oxali treatment can also enhance NF-kB activity via mitochondrial stress, whose presence in Myc-CaP cells is suggested by increased mitochondrial density and appearance of fragmented mtDNA in the cytosol. Ablation of VDAC1, through which mtDNA exits the mitochondrion (48), reduced p300 and RelA mRNA expression and abrogated induction of NLRCS and different MHC-I AgPPM components. NF-kB is also needed for induction of IFNγR2. Although IFNγR2 ablation had no effect on Oxali-induced MHC-I surface expression in cultured cells, it abrogated the rejection of Myc-CaP tumors, and inhibited induction of MHC-I AgPPM genes in mice treated with anti-PD-L1 + Oxali. We postulate that IFNγR2 induction in Myc-CaP cells makes them more responsive to IFNγ secreted by tumor-infiltrating CTLs.

MARKERs of NCs and Immunological Inflammation

**Fig. 8. Oxali-enhanced immune rejection requires NF-kB signaling.** (A) Mice bearing s.c. Myc-CaP tumors generated by control and RelA-silenced cells were treated and analyzed as described in Fig. 7A. Each dot represents a treatment group mean ± SEM. (B) Single tumor cell suspensions were analyzed for H-2Kb (Left) and H-2Dd (Right) expression on CD45 cells. (C) Scheme of vaccination experiments (Left). Two groups of mice were immunized with lysates of Oxali-killed shRNA-ctrl (MCnt) or p300-silenced (MC-p300) Myc-CaP cells. After 7 d, mice were s.c. inoculated with live shRNA-ctrl (MCnt) cells. Live shRNA-ctrl (MCnt) cells were also implanted into nonimmunized mice as a control. Tumor growth curves are shown (Right). (A–C) Two-sided t test (mean ± SEM) and two-way ANOVA were used to determine significance. *P < 0.05; **P < 0.01; ***P < 0.001; NS, not significant. Specific n values are shown in A–C.

**Materials and Methods**

Detailed information about the animal models, in vivo and in vitro studies, flow cytometry, qRT-PCR, immunoblot analysis, bioinformatic analysis, statistics, and materials is provided in SI Appendix, Material and Methods and Table S2.

**Data Availability.** This study did not generate new unique materials. The sequencing data are available in National Center for Biotechnology Information’s Gene Expression Omnibus (GEO) database: mouse ATAC-seq (GSE126287) and RNA-seq (GSE126274). Data from in vitro experiments are available under the GEO accession no. GSE126288. Single-cell RNA-seq data from live CD8+CD3+ tumor-infiltrating cells are available under the GEO accession no. GSE151611. The results shown here are in part based upon data generated by the TCGA Research Network: https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/ctga.

**ACKNOWLEDGMENTS.** We thank N. T. Ryujin, A. Perkins, C. R. Lichtenstein, T. Deerinck, M. Mackey, D. T. Tam, S. Lee, E. Sanchez-Lopez, S. Pandit, K. Wong, and M. Muldorl for technical support and help; and A. Birmingham, L. Delamarre, E. Fokas, and C. M. Rödel for discussions and advice. S.S. was supported by Prostate Cancer Foundation Young Investigator Award, Merck Investigator Studies Program 57917, and the National Institute on Alcohol Abuse and Alcoholism funded Southern California Research Center for Alcohol Liver and Pancreatic Diseases & Cirrhosis (P50 AA011999). Work in the M.K. laboratory was supported by grants from the NIH (R01AI03477, CA128814, and CA211794), the Tower Cancer Research Foundation, San Diego National Cancer Institute’s Cancer Centers Council (C3), and Padres Pedal the Cause No. PTC2018. Additional support came from U01AA027681 (to S.S. and M.K.), P01 CA128814 (to M.K./Ze Ronai [Cancer Center, Sanford Cancer Centers Council (C3), and Padres Pedal the Cause]).
1. A. M. M. Eggermont et al., Adjuvant pembrolizumab versus placebo in resected stage III melanoma. *N. Engl. J. Med.* 378, 1789–1801 (2018).
2. L. Gandhi et al.; KEYNOTE-189 Investigators, Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N. Engl. J. Med.* 378, 2078–2092 (2018).
3. W. H. Hugo and genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* 165, 35–46 (2016).
4. J. J. Havel, D. Chowell, T. A. Chan, The evolving landscape of biomarkers for check-point inhibitor monotherapy. *Nat. Rev. Cancer* 19, 133–150 (2019).
5. F. Forfoni et al., Cancer immuno-therapy efficacy and patients’ sex: A systematic review and meta-analysis. *Lancet Oncol.* 19, 737–746 (2018).
6. S. Guo, M. Contratto, G. Miller, L. Leichman, J. Wu, Immuno-therapy in pancreatic cancer: Unleash its potential through novel combinations. *World J. Oncol.* 8, 230–240 (2017).
7. E. S. Antonarakis et al., Pembrolizumab for treatment-refractory metastatic castration-resistant prostate cancer: Multicohort, open-label phase II KEYNOTE-199 study. *J. Clin. Oncol.* 38, 395–405 (2020).
8. M. K. Hossain, K. Nahar, O. Donkor, V. Apostolopoulos, Immune-based therapies for metastatic prostate cancer: An update. *Immunotherapy* 10, 283–298 (2018).
9. T. M. Beer et al., Randomized, double-blind, phase III trial of ipilimumab versus placebo in asymptomatic or minimally symptomatic patients with metastatic chemotherapy-naïve castration-resistant prostate cancer. *J. Clin. Oncol.* 35, 40–47 (2017).
10. S. Mariahasan et al., TGFβ attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* 554, 544–548 (2018).
11. N. E. Antonarakis et al., Pembrolizumab monotherapy for metastatic castration-resistant prostate cancer: An update. *Immunotherapy* 10, 283–298 (2018).
12. D. D. Chen, J. Healy, J. Yewdell, A. W. Young, HLA-A2 specific peptides: MHC class I-based cancer immunosurveillance and immunoevasion. *Nat. Rev. Immunol.* 15, 1038–1057; 020–0390-6 (2020).
13. S. Yoshishima et al., NLRC5/MHC class I transactivator is a target for immune evasion in cancer. *Proc. Natl. Acad. Sci. U.S.A.* 113, 5999–6004 (2016).
14. T. N. Schumacher, W. Schepers, P. Kvistborg, Cancer neoantigens. *Annu. Rev. Immunol.* 37, 173–200 (2019).
15. L. Galiuzzi, A. Buqué, O. Kepp, L. Zitzová, G. Kroemer, Immunogenic cell death in addiction to starvation disease. *Nat. Rev. Immunol.* 17, 97–111 (2017).
16. L. Galiuzzi et al., Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. *J. Immunother.* 8, e000337 (2020).
17. D. S. Chen, I. Millman, Elements of cancer immunity and the cancer-immune set point. *J. Exp. Med.* 215, 297–306 (2018).
18. D. Chowell et al., Evolutionary divergence of HLA class I genotype impacts efficacy of cancer immunotherapy. *Nat. Med.* 25, 1715–1720 (2019).
19. S. Gettering et al., Impaired HLA class I antigen processing and presentation as a mechanism of acquired resistance to immune checkpoint inhibitors in lung cancer. *Cancer Discov.* 7, 1420–1435 (2017).
20. M. Smahel, PD-1/PD-L1 blockade therapy for tumors with downregulated MHC class I expression. *Int. J. Mol. Sci.* 18, 1331 (2017).
21. N. Vörös, S. Neen, D. R. Siemens, M. Kötő, The tumor immune contexture of prostate cancer. *Front. Immunol.* 10, 603 (2019).
22. P. Cresswell, A. L. Ackerman, A. Giocondo, D. R. Peaper, P. A. Wearsch, Mechanisms of MHC class I restricted antigen processing and cross-presentation. *Immunol. Rev.* 263, 145–157 (2015).
23. S. Shalapour et al., Immunosuppressive plasma cells impede T-cell-dependent immunogenic nature. *Science* 321, 94–98 (2015).
24. C. He et al., Core-shell nanoscale coordination polymers combine chemotherapy and photodynamic therapy to potentiate checkpoint blockade cancer immunotherapy. *Nat. Commun.* 7, 12499 (2016).
25. C. Pirschke et al., Immunogenic chemotherapy sensitizes tumors to checkpoint blockade therapy. *Immunity* 44, 343–354 (2016).
26. D. S. Chen, I. Millman, Oncology meets immunology: The cancer-immunity cycle. *Immunity* 39, 1–10 (2013).
27. J. D. Buenrostro, B. Wu, H. Y. Chang, W. J. Greenleaf, ATAC-seq: A method for assessing chromatin accessibility genome-wide. *Curr. Protoc. Mol. Biol.*, 10.21.81–10.21.99 (2015).
28. S. C. Wei et al., Fundamental mechanisms of immune checkpoint therapy blockade. *Cancer Discov.* 8, 1069–1086 (2018).
29. N. Karachalios et al., Interferon gamma, an important marker of response to immune checkpoint blockade in non-small cell lung cancer and melanoma patients. *Ther. Adv. Med. Oncol.* 10, 175883401774948 (2018).
30. T. A. Hore, J. E. Deakin, J. A. Marshall Graves, The evolution of epigenetic regulators CTCF and BORIS/CTCFL in amniotes. *PLoS Genet.* 4, e1000169 (2008).
31. D. Bernal, S. Pourciau, M. J. D. H. Beach, M. C. Koh et al., MYC confers androgen-independent prostate cancer cell growth. *J. Clin. Invest.* 112, 1724–1731 (2003).
32. C. M. Koh et al., MYC and prostate cancer. *Genes Cancer* 1, 617–628 (2010).
33. C. A. Heinlein, C. Chang, Androgen receptor in prostate cancer. *Endocr. Rev.* 25, 276–308 (2004).
34. K. M. F. Jin, P41G1M103412, 24G1M137200, and 5100DD21784 (to N.I. and M.J. Mary Kay Ash Breast Cancer Grant 047,16, and Moore Cancer Center Award (to S.B. and J. E. Dixon) (Department of Pharmacology, University of California San Diego).