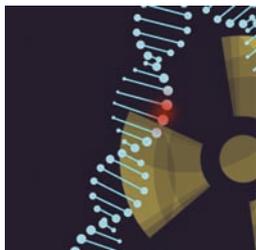


Mutations in *KEAP1* or *NFE2L2* Predict Radioresistance in Lung Cancer

- *KEAP1* or *NFE2L2* mutations were associated with local recurrence after radiotherapy in non-small cell lung cancer.
- Only pathogenic loss-of-function *KEAP1* mutations or gain-of-function *NFE2L2* mutations conferred this risk.
- *In vitro*, glutaminase-inhibitor treatment sensitized *KEAP1*-mutant lung cancer cells to radiation.



Although tumor genotyping is not typically performed for patients with non-small cell lung cancer (NSCLC) undergoing radiotherapy, recent evidence suggests that activation of the *KEAP1*-*NFE2L2* stress-response pathway may promote radioresistance and that *KEAP1* or *NFE2L2* (also known as *NRF2*) mutations may

be associated with risk of local recurrence. In a study including 232 patients with NSCLC treated with radiotherapy or surgery with curative intent, Binkley, Jeon, and colleagues found that mutations in *KEAP1* or *NFE2L2* predicted local recurrence after radiotherapy but not surgery. However, 10 out of 17 patients with *KEAP1* or *NFE2L2* mutations did not exhibit local recurrence following radiotherapy, suggesting that some mutations in these genes do not confer increased risk. Indeed, only loss-of-function *KEAP1* mutations or gain-

of-function *NFE2L2* mutations (collectively referred to as pathogenic mutations) were strongly associated with local recurrence after radiotherapy. Some tumors with risk-associated pathogenic *KEAP1* or *NFE2L2* mutations did not recur, but these tumors tended to be smaller pretreatment. Interestingly, expression of *NFE2L2* target genes was not predictive of local recurrence following radiotherapy. Notably, recent studies have identified glutamine metabolism as a key cellular dependency following *KEAP1* loss, leading to susceptibility to glutaminase inhibition. Correspondingly, treatment with a glutaminase inhibitor sensitized otherwise radioresistant *KEAP1*-mutant lung cancer cells to radiation *in vitro*. Collectively, these findings demonstrate the relationship between pathogenic *KEAP1* or *NFE2L2* mutations and local recurrence of NSCLC following radiotherapy and suggest that glutaminase inhibition may be a useful strategy to overcome this resistance. ■

See article, p. 1826.

ctDNA Analysis Has Prognostic Value for Immune Checkpoint Blockade

- Circulating tumor DNA (ctDNA) was analyzed in patient samples from immune checkpoint blockade (ICB) trials.
- A >50% decrease in the ratio of on-treatment to pretreatment ctDNA variant allele frequency distinguished response.
- Noninvasive ctDNA analysis may complement existing techniques for evaluating prognosis in patients receiving ICB.



Characteristics of circulating tumor DNA (ctDNA) in patients with cancer have been assessed as potential biomarkers in a variety of clinical contexts; however, information regarding whether ctDNA evaluation can aid prognostic determination in patients undergoing immune checkpoint blockade (ICB) is lacking. Zhang,

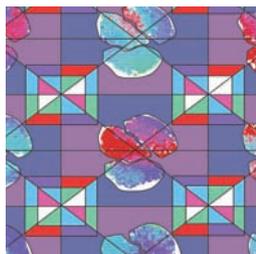
Luo, and colleagues analyzed variant allele frequency (VAF) in ctDNA samples (978 pretreatment; 171 on treatment) from patients with cancer representing sixteen advanced solid-tumor types obtained during phase I/II clinical trials of the PD-L1 antibody durvalumab with or without the CTLA4 antibody tremelimumab. ctDNA was detectable in 83.2% of pretreatment samples, and this proportion varied with tumor type, with glioblastoma multiforme having the lowest detection rate (21.4%) and small-cell lung cancer and

nasopharyngeal carcinoma having the highest detection rate (>95%). The ctDNA VAF of pretreatment samples carried prognostic value: Specifically, across all tumor types, low pretreatment ctDNA VAF was associated with longer overall survival, although pretreatment ctDNA VAF was not significantly associated with objective response to ICB. In contrast, on-treatment ctDNA VAF was correlated with objective response, and the best overall survival was seen in patients with full ctDNA clearance on treatment. A decrease in the ratio of on-treatment to pretreatment VAF of 50% or greater was found to predict ICB response, and this newly designated “molecular response” was able to identify future benefit from ICB in patients initially deemed to have stable disease based on radiologic response criteria. In summary, this work shows that ctDNA analysis may serve as a useful, noninvasive complementary strategy for prognostic determination in patients receiving ICB. ■

See article, p. 1842.

NKG2A Expression Is Linked to Poor Response to NK-Cell Therapy in AML

- Cytokine-stimulated memory-like natural killer (NK)-cell therapies are being tested for acute myeloid leukemia.
- Treatment efficacy was lower for memory-like NK cells that had high expression of the inhibitory receptor NKG2A.
- This reveals one possible reason that some patients have good responses while others' disease does not respond.



Adoptive cell therapies involving transfer of natural killer (NK) cells are emerging treatments for acute myeloid leukemia (AML), and recent work has shown that NK cells can be stimulated by the cytokines IL12, IL15, and IL18 to develop memory-like characteristics that may be beneficial for such therapies. Berrien-Elliott

and colleagues performed a mass cytometry analysis on donor NK cells immediately after isolation, after 12 to 16 hours of cytokine activation (just prior to infusion), and seven days after infusion in patients, revealing that the memory-like NK cells present after seven days were distinct from baseline and post-activation NK cells. Similar populations of donor memory-like NK cells present one week following infusion were observed in the peripheral blood and bone marrow of

recipients, indicating trafficking of these NK cells to the bone marrow. *Ex vivo* coculture experiments demonstrated that the donor memory-like NK cells produced more IFN γ and MIP1 α (a chemokine) in response to exposure to human leukemia cells than unstimulated NK cells. Patients who had donor memory-like NK cells expressing high levels of the inhibitory immune checkpoint receptor NKG2A or CD8 α were less likely to benefit from treatment than patients whose donor memory-like NK cells expressed the receptor at lower levels. However, baseline expression of NKG2A by donor NK cells was not correlated with response, and further analysis showed that NKG2A was upregulated at the transcriptional level in highly NKG2A-expressing memory-like NK cells. Collectively, these findings reveal possible reasons for treatment resistance in patients with AML receiving cytokine-activated NK-cell therapies. ■

See article, p. 1854.

Degradation of B7-H4 Enhances Immunogenicity of Immune-Cold Tumors

- B7-H4, elevated in immune-cold breast cancers, was glycosylated, preventing ubiquitination and degradation.
- An eIF2 α -PERK-calreticulin axis was shown to underlie the immunosuppressive effects of B7-H4 elevation.
- Blocking B7-H4 glycosylation synergized with a doxorubicin analogue and anti-PD-L1 to prevent tumor growth.



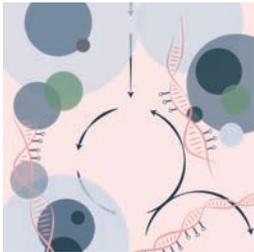
The use of immunotherapies such as immune checkpoint blockade has transformed the treatment of many cancers; however, the treatment of immune-cold tumors has remained challenging. Song, Zhou, Li, and colleagues found that immune-cold human triple-negative breast cancer (TNBC) tumors had elevated levels of B7-H4 (also known as VTCN1), a transmembrane immune checkpoint protein that inhibits T-cell effector function. *In vitro* experiments revealed that B7-H4 underwent N-linked glycosylation, and highly glycosylated B7-H4 had a substantially longer half-life than B7-H4 with low or no glycosylation; this was shown to be due to decreased ubiquitination (and thus reduced proteasomal degradation) of highly glycosylated B7-H4. Further analyses revealed that the glycosyltrans-

ferases STT3A and UGGG1 carried out B7-H4 glycosylation, whereas the ubiquitin E3 ligase AMFR was responsible for B7-H4 ubiquitination. When present at elevated levels, B7-H4 prevented immunogenic cell death by binding the translation factor eIF2 α , preventing its phosphorylation (and resulting activation) by the ER-resident stress kinase PERK and therefore blocking eIF2 α -mediated display of the “eat me” signal protein calreticulin on cancer-cell surfaces; this effect of B7-H4 was blocked by treatment with the chemotherapy drug doxorubicin. Notably, experiments using *in vivo* models of TNBC, a combination of the small-molecule inhibitor of B7-H4 glycosylation NGI-1 with the non-cardiotoxic doxorubicin analogue camsirubicin reduced tumor growth, an effect amplified by the addition of anti-PD-L1. Together, these results identify B7-H4 as a potential target in immune-cold tumors and uncover the mechanism by which it blocks antitumor immunity. ■

See article, p. 1872.

MTHFR Mediates Response to BET Inhibition in Acute Myeloid Leukemia

- Decreased function of the folate cycle enzyme MTHFR reduced BET-inhibitor sensitivity in acute myeloid leukemia.
- *In vitro*, supplementation with the MTHFR product 5-methyltetrahydrofolate restored BET-inhibitor sensitivity.
- *MTHFR* mutations that reduce enzymatic function are common, so these findings may have clinical implications.



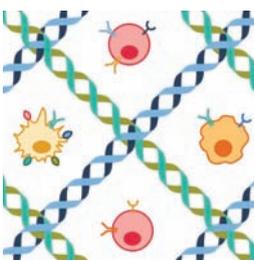
Drugs such as BET, CDK7, and CDK9 inhibitors, which dampen oncogenic *MYC* upregulation, are currently in phase I and II clinical trials for advanced solid tumors and hematologic cancers. However, it is not clear how to determine which patients are most likely to benefit and what other treatments may synergize with these drugs. Su, Ling, and colleagues found that folate starvation reduced the sensitivity of acute myeloid leukemia (AML) cell lines to BET and CDK7 inhibitors, and dietary folate restriction promoted resistance to BET-inhibitor treatment in a mouse model of AML. Supporting this finding, knockdown of *MTHFR* (encoding the rate-limiting enzyme in the folate cycle) increased BET-inhibitor resistance in human AML cells *in vitro*, and an *Mthfr*-knockdown mouse model of AML exhibited reduced sensitivity to BET

inhibition. In AML cell lines and primary AML cells, common (present in approximately 10% of Caucasian individuals) *MTHFR* mutations that reduce enzymatic function were associated with increased BET-inhibitor resistance that could be reversed via supplementation of the MTHFR product 5-methyltetrahydrofolate; similar results were observed in *Mthfr*^{-/-} mice. Correspondingly, overexpression of wild-type *MTHFR* increased AML-cell sensitivity to BET inhibitors *in vitro*. Mechanistically, disruption of the folate cycle caused increased levels of S-adenosylhomocysteine, reduced histone 3 methylation at lysine residues 27 and 9, and activation of a SPI1-mediated transcriptional program upon BET-inhibitor treatment, limiting BET-inhibitor efficacy. Collectively, these results suggest that *MTHFR* allelic status may affect response to BET inhibition and that 5-methyltetrahydrofolate supplementation may be of use in patients with *MTHFR* mutations. ■

See article, p. 1894.

The Histone Methyltransferase KMT2D Modulates Response to Anti-PD-1

- Loss-of-function mutations in *Kmt2d* increased anti-PD-1 response in genetically engineered mouse models.
- *Kmt2d* mutation caused increased immune-cell infiltration in tumors and widespread changes in tumor cells.
- *KMT2D* mutation, common in human cancers, may be a useful biomarker for predicting immunotherapy response.



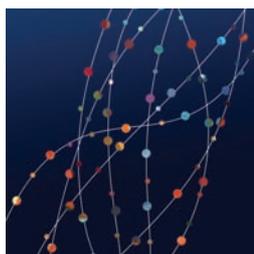
Although some factors influencing response to immune checkpoint blockade have been identified, many contributors remain unknown. To better understand resistance, Wang, Chow, Zhu, Bai, and colleagues used CRISPR-Cas9-based genome editing to generate genetically engineered mouse models (GEMM) of a variety of cancer types. In a liver cancer GEMM, loss-of-function mutations in *Kmt2d*—a frequently mutated gene in human cancers that encodes a histone 3 lysine residue 4 methyltransferase—were associated with increased response to immune checkpoint blockade using anti-PD-1. Similar results were observed for bladder cancer, triple-negative breast cancer, melanoma, and lung cancer. Dendritic-cell, macrophage, CD45⁺ immune-cell, and CD4⁺, CD8⁺, and CD8⁺IFN γ ⁺ T-cell infiltration was greater in *Kmt2d*-mutant lung tumors, and infiltration by these cells

increased upon anti-PD-1 treatment. *Kmt2d* mutation resulted in increased DNA damage in tumor cells; correspondingly, analysis of data from The Cancer Genome Atlas revealed that *KMT2D*-mutant human tumors had higher-than-average tumor mutation burden and that patients with *KMT2D*-mutant bladder cancer were more likely to respond to anti-PD-1 treatment. *Kmt2d* mutation had numerous effects on liver cancer cells, including global chromatin remodeling, large-scale transcriptomic alterations, increased intron retention, activation of transposable elements, and increased proteasomal degradation of proteins. Any of these mechanisms may explain why *Kmt2d* mutation sensitized tumors to anti-PD-1; for example, transposable-element activation may cause DNA damage and therefore lead to increased neoantigen production. In summary, this work identifies *KMT2D* mutation as a potential biomarker for anti-PD-1 response and provides explanations for this mutation's effects in cancer. ■

See article, p. 1912.

AML Driver Mutations Cause Epigenetic Diversity before Leukemogenesis

- Somatic driver mutations of acute myeloid leukemia (AML) were associated with epigenetic alterations in patients.
- Mouse studies showed that AML driver mutations caused diversity of epigenetic alleles prior to leukemogenesis.
- Epigenetic therapies reduced epigenetic-allele diversity *in vivo*, showing that this state is targetable.



Some acute myeloid leukemias (AML) are characterized by high diversity of epigenetic alleles—or “epialleles,” a term that refers to epigenetic variants defined by the CpG dinucleotide methylation pattern at given loci. This trait is linked to poor prognosis, but its origins are not well understood. To identify the

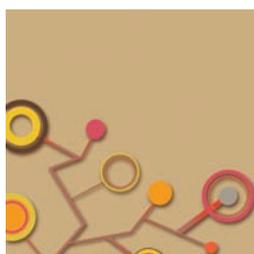
cause of epiallelic diversity in AML, Li, Chen, and colleagues began by analyzing CpG methylation profiles of 119 patients with AML harboring common AML driver mutations, finding that certain driver mutations were associated with specific epiallelic patterns and that epiallelic diversity was linked to poor clinical outcomes. Across all AML subtypes, bone marrow from patients with AML exhibited greater epiallelic heterogeneity than normal control bone marrow. In

Lin⁻Sca1⁺cKit⁺ cells from preleukemic mice harboring various leukemia driver mutations alone or in combination, double mutations were associated with greater epiallelic diversity than single mutations, and *Idh2*^{R140Q}-knock-in mice had more substantial methylomic perturbation than *Tet2*^{-/-} mice. Additionally, mice with leukemia driven by *Tet2* and *Idh2* mutations exhibited lower epiallelic diversity when treated with epigenetic therapies, including a DNA methyltransferase inhibitor and a mutant-IDH2 inhibitor. Further, in mouse leukemia models, epiallelic heterogeneity was linked to transcriptional heterogeneity, demonstrating the functional relevance of the observed epigenetic alterations. Taken together, these findings suggest that epiallelic diversity results from common somatic leukemia driver mutations, precedes leukemic transformation, and is targetable using epigenetic therapies. ■

See article, p. 1934.

New Approach Finds RAS Interactors and Synthetic-Lethal Relationships

- A new method combines affinity purification-mass spectrometry with CRISPR-Cas9-based dual-knockout screens.
- This technique uncovered interactors with RAS-family proteins in mutant *KRAS*-driven lung adenocarcinoma cells.
- Experiments using *in vitro* and *in vivo* models confirmed the functional relevance of these interactions.



Hyperactivation of RAS pathways is common in cancer, and although many regulators and effectors of RAS-family proteins have been discovered, conventional screens may fail to identify RAS interactors with paralogous proteins that can compensate for their loss. To address this, Kelly, Kostyrko, Han, and colleagues developed a method using affinity purification-mass spectrometry analyses to capture and pinpoint proteins that interact with RAS-family proteins, then performed the analyses again with the identified proteins to find more interactors in an iterative fashion and develop a protein-protein interaction network. Then, the genes encoding the proteins in this network were subjected to CRISPR-Cas9-based dual-knockout screens in mutant *KRAS*-driven lung adenocarcinoma cell lines to detect synthetic-lethal interactions. Using

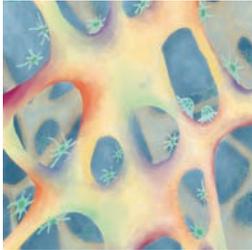
this new approach, the endocytosis regulator RIN1 and the cell-adhesion mediator RADIL were found to be effectors of GTP-bound (active) KRAS, and *in vitro* experiments showed that these effectors enhanced cancer-fueling macropinocytosis and cell migration. Many synthetic-lethal interactions were also uncovered, notably among paralogous members of the MAP kinase pathway and other KRAS effectors. An unexpected interaction was found between *RAP1GDS1*, encoding a guanine nucleotide exchange factor that stimulates small GTPases, and *RHOA*, encoding a small GTPase in the RAS superfamily that is a *RAP1GDS1* target. This synthetic-lethal interaction was confirmed in a mutant *KRAS*-driven lung adenocarcinoma model *in vivo*. In summary, this work demonstrates that this approach—which can be applied to many cancer-linked proteins—can identify previously unknown protein-protein interactions and synthetic-lethal gene combinations of functional relevance. ■

See article, p. 1950.

See article, p. 1950.

Histone 3.3 Mutation Causes Epigenetic Remodeling Underlying GCTB

- An H3.3 mutation found in most cases of giant cell tumor of bone exerted *cis* and *trans* effects on the epigenome.
- This broad epigenetic remodeling caused altered cell-fate decisions that may underlie osteoclast recruitment.
- These findings provide an explanation for how a single mutation can drive development of this tumor type.



Giant cell tumor of bone (GCTB) is composed of destructive multinucleated osteoclast-like cells, macrophage-like monocytes, and neoplastic stromal cells that harbor histone 3.3 (H3.3) mutations at glycine residue 34 (most commonly G34W) in almost all cases. To investigate the effects of H3.3 G34W mutations,

Khazaei, De Jay, Deshmukh, Hendrikse, and colleagues began by using CRISPR–Cas9-based genome editing to modify the mutant allele encoding H3.3^{G34W} (*H3F3A*^{G34W}) in stromal cell lines derived from primary GCTBs, generating cell lines in which the mutant allele had either loss-of-function insertions or deletions or was corrected to the wild-type variant. Orthotopic implantation experiments established that the mutant *H3F3A*^{G34W} allele was required for GCTB growth and increased osteoclast recruitment. H3.3^{G34W} was found predominantly in H3.3-enriched euchromatic regions, where

it affected chromatin in *cis*, but the mutant histone also exerted effects in *trans*, with its expression being associated with redistribution of wild-type H3.3. This may have been related to the observed redistribution of a repressive chromatin mark [histone 3 lysine residue 27 trimethylation (H3K27me3)] from intergenic to gene-containing regions, possibly due to loss of the antagonistic active-chromatin mark H3K36me3 as a consequence of the G34W mutation. The resulting transcriptional aberrations were associated with altered cell-fate decisions in mesenchymal progenitors, blocking their differentiation. GCTB stromal cells carrying the G34W mutation resembled osteoblast-like progenitors skewed toward myofibroblast-like cells that secrete extracellular-matrix proteins predicted to attract osteoclasts. In summary, epigenetic remodeling driven by G34W-mutant H3.3 promotes differentiation defects that lead to aberrant recruitment of giant osteoclasts by neoplastic stromal cells in GCTB. ■

See article, p. 1968.

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