The detection of circulating tumor DNA (ctDNA) has been shown to predict recurrence of cancers such as colorectal and breast cancers. To ascertain whether ctDNA profiling can reliably identify minimal residual disease (MRD) in patients with lung cancer, Chaudhuri and colleagues performed deep sequencing for the presence of 128 recurrent lung cancer–associated driver and passenger mutations on pre- and post-treatment blood and tissue samples from 40 patients with localized lung cancer who received surgery or radical radiotherapy. ctDNA was detected in the baseline samples from 37 of 40 (93%) patients and in the post-treatment samples from 20 of 37 (54%) evaluable patients. ctDNA detection preceded detection of RECIST 1.1 progression by a median of 5.2 months, and MRD landmark analysis at ≥4 months post-treatment identified ctDNA in 17 of 32 (53%) evaluable patients. Further, tracking single mutations in ctDNA resulted in the identification of MRD in 23 of 40 (53%) patients, suggesting that tracking multiple mutations enhances the sensitivity of MRD detection in patients with lung cancer. Freedom from progression at 36 months was exhibited by none of the patients with detectable ctDNA at the MRD landmark and by 15 of 32 (93%) patients with undetectable ctDNA at the MRD landmark (hazard ratio 43.4, 95% CI 5.7-341). Together, these results show that increased sensitivity of ctDNA detection in patients with localized lung cancer reliably predicts recurrence and may provide insight into potential targeted therapeutic approaches.

See article, p. 1394.

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Lymphodepletion chemotherapy followed by autologous transfer of CD19-targeted chimeric antigen receptor-modified T (CAR-T) cells has achieved responses in patients with refractory CD19+ B-cell acute lymphoblastic leukemia (B-ALL), chronic lymphocytic leukemia (CLL), and non-Hodgkin lymphoma (NHL). However, many patients treated with CD19 CAR-T cells develop cytokine release syndrome (CRS), a systemic inflammatory response frequently accompanied by neurologic adverse events. Gust, Hay, Hanafi, and colleagues investigated neurologic adverse events in 133 patients with refractory B-ALL, NHL, or CLL who had received chemotherapy and CD19 CAR-T cell infusion. Twenty-eight of the 133 patients (21%) developed one or more grade 3+ neurologic adverse events. CRS preceded neurotoxicity in all patients who developed grade 3+ neurotoxicity, and neurotoxicity was fatal in 4 (3%) patients. Twenty-three of the patients exhibiting neurotoxicity underwent brain MRI, and acute abnormalities were seen in 7 of 23 patients (30%), 4 of whom had fatal neurotoxicity, suggesting that an abnormal MRI may be associated with poor outcomes. Further, severe neurotoxicity was associated with endothelial activation, including increased blood–brain barrier permeability, allowing systemic cytokines including IFNy to enter the cerebrospinal fluid, inducing brain vascular pericyte stress and the secretion of endothelium-activating cytokines. The brain of a patient who died of CRS-induced neurotoxicity showed signs of endothelial activation and vascular disruption, and endothelial activation prior to treatment was linked to an increased risk of high-grade neurotoxicity. Collectively, these findings identify risk factors for neurotoxicity following CD19 CAR-T cell therapy, and suggest that endothelial activation may serve as a biomarker for severe neurotoxicity.

See article, p. 1404.
Immune checkpoint inhibitors (ICI) have demonstrated clinical activity in patients with non–small cell lung cancer. However, the benefit of ICIs is limited by acquired resistance, and the mechanisms underlying the development of resistance remain poorly understood. To identify potential mechanisms of resistance, Gettinger, Choi, Hastings, and colleagues assessed the genomic, transcriptomic, and inflammation landscapes of 14 lung tumors resistant to ICIs and generated patient-derived xenografts (PDX) from 3 of these tumors. In 6 of 8 paired cases, there was an increase in somatic nonsynonymous mutations after acquired ICI resistance. Copy-number analysis revealed a homozygous loss of Beta-2 microglobin ($B2M$), which is required for HLA Class I antigen processing, that resulted in loss of cell-surface HLA Class I expression in a tumor with acquired resistance to ICIs and heterozygous loss in a second patient. $B2M$ was also lost in the corresponding PDX and downregulated in two additional ICI-resistant PDXs. Deletion of $B2M$ in a syngeneic immunocompetent mouse model of lung cancer conferred resistance to anti–PD-1 therapy, impairing CD8$^+$ T cell–mediated cytotoxicity, and resulting in tumor growth despite anti–PD-1 therapy. The ICI-resistant tumors had an inflammatory microenvironment characterized by upregulation of immune inhibitory receptor genes (including LAG3, PD-1, TIGIT, 2B4, CTLA4, and TIM3) and increased expression of CD8$^+$ T-cell effector molecules (including GZMB, TNFα, and IFNγ). Taken together, these findings suggest that disruption of HLA Class I antigen processing and presentation may promote acquired resistance to ICI in patients with lung cancer.

See article, p. 1420.

Loss of Primary Cilia Promote Resistance to SMO Inhibitors

• A transposon screen found that disrupting the ciliogenesis gene $Ofd1$ induces SMO inhibitor resistance.
• Ciliary gene mutations are linked to SMO inhibitor resistance in patients with basal cell carcinoma.
• Cilia loss protects tumor cells from susceptibility to SMO inhibitors by maintaining low HH signaling.

Aberrant hedgehog (HH) signaling is common in medulloblastoma and basal cell carcinoma (BCC) and is often caused by inactivating mutations in $PTCH$, which encodes the HH receptor. Loss of $PTCH$ function results in trafficking of SMO to the primary cilia, triggering signaling that results in nuclear import of GLI transcription factors to promote transcription of target genes that drive tumor growth. SMO inhibitors have emerged as anticancer agents, but the mechanisms driving resistance to SMO inhibitors have not been fully elucidated, prompting Zhao, Pak, and colleagues to perform a genome-wide transposon mutagenesis screen in HH signaling–dependent medulloblastoma cells to identify alterations that promote resistance to SMO inhibitors. The ciliogenesis gene $Ofd1$, which is required for formation of the primary cilium, emerged as a top hit. $Ofd1$ disruption induced resistance to SMO inhibition. Similarly, loss of other essential ciliogenesis genes also conferred specific resistance to SMO inhibitors, suggesting that primary cilia loss induces SMO inhibitor resistance. SMO inhibition resulted in conversion of much of the full-length GLI2 to the truncated repressor form (GLI2-R). However, loss of primary cilia eliminated GLI2-R formation and prevented SMO-dependent activation of HH signaling, resulting in low basal levels of GLI2 activity that allowed cells to evade SMO inhibition. In vivo, OFD1-mutant tumors were resistant to SMO inhibition, and, in patients with BCC, mutations in ciliary genes were associated with resistance to SMO inhibitors. Altogether, these findings reveal that loss of primary cilia can promote resistance to SMO inhibitors.

See article, p. 1436.
mTOR and HDAC Inhibitors Enhance Oxidative Stress in RAS-Driven Tumors

Effective therapeutic strategies for tumors driven by mutations or excessive activation of RAS, such as NF1-deficient malignant peripheral nerve sheath tumors (MPNST), have yet to be identified. The mTOR pathway has been identified as a critical regulator of the glutathione antioxidant pathway in NF1-mutant MPNSTs; however, treatment with mTOR inhibitors results only in cytoprotic reactive oxygen species (ROS) levels in tumors, cooperated with mTOR inhibitors to further enhance ROS levels and trigger catastrophic oxidative stress in human MPNST cells, resulting in induction of cell death and tumor regression in a genetically engineered mouse model. In addition to suppression of the glutathione pathway by mTOR inhibitors, dual blockade of HDAC and mTOR converges to inhibit the thioredoxin antioxidant pathway. This inhibitory effect was mediated by cooperative induction of thioredoxin-interacting protein (TXNIP) via HDAC inhibitor–driven effects on chromatin near the TXNIP transcription start site and mTOR inhibitor–driven activation of the MONDOA–MLX transcription complex. HDAC/mTOR inhibitor–stimulated cell death was dependent on suppression of thioredoxin as well as TXNIP-mediated activation of apoptosis signal–regulating kinase 1 (ASK1). Of note, treatment with HDAC and mTOR inhibitors also induced tumor regression in NF1- and KRAS-mutant non–small cell lung cancer (NSCLC) models. These results suggest that selective enhancement of oxidative stress by HDAC/mTOR inhibitors may represent a promising and broadly applicable therapeutic strategy for RAS-driven tumors.

See article, p. 1450.

Galectin-3 May Be A Druggable Target in KRAS-Mutant Tumors

KRAS is recruited by cell surface receptors into cell membrane nanoclusters that promote downstream signaling in matrix-adherent cells, but cell surface receptors cluster more poorly in cells that exhibit anchorage-independent growth. Integulin αvβ3 does cluster on nonadherent tumor cells, and it has been shown to interact with the carbohydrate-binding protein galectin-3 to promote KRAS clustering in nonadherent cells, prompting Seguin and colleagues to hypothesize that expression of integrin αvβ3/galectin-3 may be essential in KRAS-mutant tumors, suggesting their potential as therapeutic targets. Indeed, KRAS-addicted lung cancer cells required integrin αvβ3/galectin-3 for anchorage-independent growth, and αvβ3-positive cells were uniquely addicted to mutant KRAS. The αvβ3-positive cells exhibited enhanced macropinocytosis when galectin-3 was also expressed, facilitating nutrient uptake to support anchorage-independent growth. Further, αvβ3-positive KRAS-mutant cells had an enhanced ability to manage oxidative stress, exhibiting low levels of mitochondrial reactive oxygen species (ROS) to facilitate anchorage-independent growth. Consistent with these findings, a galectin-3 inhibitor (GCS-100) that blocked the interaction with αvβ3 selectively killed KRAS-addicted lung and pancreatic cancer cells in vitro and suppressed the growth of lung and pancreatic tumors in vivo in xenografts and patient-derived xenografts. By blocking the interaction with αvβ3, GCS-100 disrupted KRAS clustering in anchorage-independent cells, thereby reducing nutrient uptake by macropinocytosis and increasing mitochondrial ROS. Collectively, these findings reveal that galectin-3 may be a druggable therapeutic target in patients with KRAS-mutant tumors, and suggest that αvβ3 expression may serve as a biomarker for susceptible tumors.

See article, p. 1464.

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