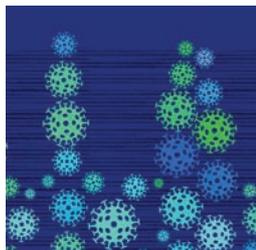


## Patients with Cancer Are Much More Vulnerable to COVID-19 Death

- Twenty-eight percent of COVID-19-positive patients with cancer in a New York hospital system died of COVID-19.
- Patients with blood cancers had higher fatality, and risk factors were age, performance status, and comorbidities.
- Patients with cancer are markedly more vulnerable to COVID-19 death regardless of cancer treatment status.



Because of immunosuppression, comorbidities, and other factors, patients with cancer may be more susceptible to death from COVID-19—and early data from China and Italy support this notion. In an analysis of the outcomes of COVID-19 infection in 218 patients with cancer in a hospital system in New York,

Mehta, Goel, Kabarriti, and colleagues found that patients with cancer indeed exhibited higher mortality than the general population. Twenty-eight percent of the 218 patients died from COVID-19, whereas early estimates suggest that only one to four percent of patients among the general population with COVID-19 will die of the disease. This finding remained significant after adjusting for age. Mortality rates exhibited a cancer type-specific trend, with death occurring

in 37% of patients with hematologic malignancies and 25% of patients with solid tumors. Among patients with solid tumors, those most vulnerable were patients with pancreatic cancer (67% mortality); lung cancer (55% mortality); or colorectal, upper gastrointestinal, or gynecologic cancer (all 38% mortality). Those with genitourinary or breast cancer, in contrast, fared better, with mortalities of 15% and 14%, respectively. The death rates in patients with solid tumors reflect the fatality rates of patients with these cancer types in general. Notably, active chemotherapy or radiotherapy was not associated with increased case-fatality rates, nor was immunotherapy, although the number of patients in this cohort receiving immunotherapy was low. This work suggests that patients with cancer exhibit strikingly elevated susceptibility to death from COVID-19 and that proactive measures should be put into place to protect this vulnerable group. ■

See article, p. 935.

## Many Infant High-Grade Gliomas May Harbor Targetable Gene Fusions

- A unique subset of infant high-grade gliomas with shared clinical and molecular characteristics was identified.
- Patients with these tumors fared better and often had fusions involving genes encoding receptor tyrosine kinases.
- *In vivo* and preliminary clinical evidence suggests that targeted drugs may be of use in fusion-positive patients.



Although the prognosis for pediatric high-grade glioma is poor, infants—typically defined as those younger than three to five years of age—tend to fare better. Clarke, Mackay, Ismer, and colleagues collected 241 tumors diagnosed as high-grade or diffuse gliomas from patients four years old or younger at

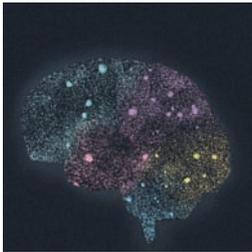
diagnosis and performed histologic analyses, methylation profiling, and custom-panel, genome, or exome sequencing. After excluding non-gliomas and gliomas matching known subtypes, 130 infant gliomas remained, comprising a set of tumors with shared clinical and molecular characteristics. These gliomas mainly occurred in the cerebral hemispheres and were typically found in the youngest patients (under one year old), whose overall survival was similar to those with lower-grade tumors. Nearly two thirds of the tumors

had fusions involving the receptor tyrosine kinase-encoding genes *ALK*, *NTRK1/2/3*, *ROS1*, or *MET*. Mice harboring tumors with the most commonly seen *ALK* gene fusion (*PPP1CB-ALK*) were treated with the *ALK* inhibitor lorlatinib, which was chosen among *ALK* inhibitors due to its superior antiproliferative effects on *PPP1CB-ALK*-mutant cells *in vitro*. In these mice, lorlatinib outperformed standard-of-care chemotherapy (temozolomide), which slowed tumor growth, whereas lorlatinib caused tumor regression and improved overall survival. Notably, one patient whose *ALK* fusion-positive tumor was evaluated in the study whose disease progressed after total resection and two chemotherapy protocols experienced stable disease for two years after targeted therapy. Collectively, these results illustrate the unique properties of infant high-grade gliomas and provide ideas for targeted treatments that may be of use. ■

See article, p. 942.

## Glioblastoma Cerebral Organoids Recapitulate Primary Glioblastomas

- This work characterizes four glioblastoma models, highlighting how they compare to primary tumors.
- Compared with other models, glioblastoma cerebral organoids (GLICO) most closely resembled primary glioblastomas.
- The transcriptomic and cell type-composition similarities were dependent on the GLICO microenvironment.



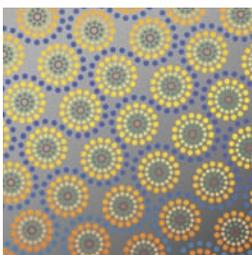
Although numerous models of glioblastoma exist, no model perfectly recapitulates the disease, and it is critical to determine which model is most appropriate to use in given circumstances. With this in mind, Pine, Cirigliano, and colleagues characterized four glioma stem cell-derived tumor models—two-dimensional glioma sphere cultures (2-D), three-dimensional tumor organoids (TO), glioblastoma cerebral organoids (GLICO), and patient-derived xenografts—from five patients with glioblastoma. With regard to microscopic invasiveness, GLICOs and xenografts behaved like glioblastomas, and both bulk transcriptomic analyses and single-cell RNA-sequencing experiments showed that, compared with 2-Ds and TOs, GLICOs and xenografts more closely resembled the primary tumors. An analysis of the cellular heterogeneity in each model revealed marked differences among them,

with GLICOs having a profile most similar to that of primary glioblastomas, including higher percentages of proneural cells and lower percentages of mesenchymal cells. Focusing on cells of glial lineages, GLICOs again showed the greatest similarity to primary glioblastomas, exhibiting retention of neural progenitor cell and oligodendrocyte progenitor cell populations that the other models largely lacked. Notably, GLICOs expressed genes that may be relevant for glioma stem cell-like behavior, such as *SOX4*, *BCAN*, and genes encoding members of the Notch pathway. Demonstrating that GLICOs' ability to recapitulate primary glioblastomas depends on the microenvironment, the transcriptomic and cell-composition overlap between GLICOs and glioblastomas was diminished when GLICO cells were replated in two-dimensional culture conditions. In summary, this work characterizes multiple commonly used models for glioblastoma research, particularly highlighting the use of GLICOs for relevant applications. ■

See article, p. 964.

## G9a Promotes Melanoma Development via WNT Pathway Activation

- *EHMT2*, encoding the histone methyltransferase G9a is amplified or has activating mutations in many melanomas.
- G9a repressed transcription of the WNT pathway inhibitor *DKK1* by modifying histones at *DKK1*'s promoter.
- This study reveals the mechanism by which G9a promotes oncogenesis, providing potential drug targets.



The histone methyltransferase G9a (encoded by *EHMT2*) acts as part of a heterodimeric complex with G9a-like protein (GLP) to mono- and dimethylate histone 3 lysine residue 9 (H3K9me1 and H3K9me2, respectively) via its catalytic SET domain, leaving transcriptionally repressive chromatin marks.

Overexpression of G9a has been linked to cancer cell proliferation, chemoresistance, metastasis, and other oncogenic properties in multiple cancers, but the specific genomic alterations of *EHMT2* responsible for promoting oncogenic pathways are unknown. In many human melanomas, Kato and colleagues discovered that *EHMT2* amplifications or mutations were present, with recurrent mutations (specifically G1069L/W) increasing G9a's affinity for H3K9me1 and, when G9a was complexed with GLP, activating G9a's catalytic SET domain. *In vitro* and *in vivo* experiments showed

that expression of G9a<sup>G1069L/W</sup> promoted melanoma development, and G9a activity was necessary for tumorigenesis in a model of melanoma driven by *EHMT2* amplification. Mechanistically, *EHMT2* amplification increased expression of *MITF*—encoding a regulator of melanocyte differentiation and development that also acts as an oncogene when mutated or amplified—via G9a-mediated repression of genes encoding WNT antagonists, leading to activation of the canonical WNT- $\beta$ -catenin signaling pathway. Specifically, G9a dimethylated H3K9 at the promoter of *DKK1*, a WNT pathway inhibitor, resulting in reduced *DKK1* mRNA and *DKK1* protein levels in *EHMT2*-amplified melanoma cells. Highlighting the broader relevance of these findings, further investigation revealed that G9a-mediated *DKK1* repression and consequent WNT pathway activation appeared to be conserved in several other cancer types. Together, these findings elucidate the mechanism by which *EHMT2* mutation or amplification can promote cancer. ■

See article, p. 980.

## CHK1 Inhibition Shows Promise in *EZH2*-Mutant T-ALL with Poor Prognosis

- Loss of function of *EZH2* is associated with poor prognosis in T-cell acute lymphoblastic leukemia (T-ALL).
- *EZH2*-mutant T-ALL has increased replication stress due to *MYCN* upregulation, sensitizing cells to CHK1 inhibition.
- This work identifies drugs showing promise *in vivo* for hard-to-treat T-ALL and provides mechanistic understanding.



Loss-of-function mutations affecting the epigenetic regulator PRC2's catalytic component *EZH2*, which trimethylates histone 3 at lysine residue 27 (H3K27me3), are common in T-cell acute lymphoblastic leukemia (T-ALL) and associated with pathogenesis and poor prognosis.

León and colleagues performed a drug screen in *EZH2*-deficient human T-ALL cells, revealing that these cells were sensitive to four structurally distinct inhibitors of CHK1, a replication stress-checkpoint protein kinase. Consistent with the specific effect of these drugs, *EZH2*-deficient T-ALL cells in which *CHEK1* (encoding CHK1) was knocked down had impaired growth. Further supporting the observed relationship between CHK1 and *EZH2*, T-ALL cells pretreated with a specific *EZH2* inhibitor exhibited heightened sensitivity to CHK1 inhibition. Mechanistically, *EZH2*-knock-

out T-ALL cells exhibited increased replication stress, increasing their dependency on the CHK1-mediated replication stress-checkpoint response. Likely because *EZH2*-knockout T-ALL cells exhibited elevated replication stress, CHK1 inhibition caused these cells to undergo apoptosis during the S and G<sub>2</sub> phases. Notably, *EZH2*-knockout T-ALL cells had a gene signature reminiscent of that of early T-cell precursor ALL, an ALL subtype with poor prognosis, and this was associated with derepression of early thymic genes, including *MYCN*, the transcription of which depended on a distal PRC2-regulated enhancer. Deletion of the *MYCN* enhancer was sufficient to reverse the replication-stress phenotype. Finally, *in vivo*, treatment of *EZH2*-mutant T-ALL with a CHK1 inhibitor reduced leukemia growth and increased survival, an effect not seen in *EZH2*-wild-type T-ALL. Together, these results identify a previously unknown vulnerability of a recalcitrant malignancy, paving the way for new treatment modalities. ■

See article, p. 998.

## Pancreatic Cancer Has a Specific Dependence on an Alanine Transporter

- Pancreatic ductal adenocarcinoma (PDAC) cells imported alanine using the amino acid transporter SLC38A2.
- Failure to import stromal alanine caused a metabolic crisis, and lack of SLC38A2 hindered PDAC growth *in vivo*.
- This work shows how PDAC cells fuel their demand for alanine and provides SLC38A2 as a potential drug target.



Alanine supplied by stromal pancreatic stellate cells (PSC) promotes pancreatic ductal adenocarcinoma (PDAC) metabolism and tumorigenicity; however, the biochemical mechanisms underlying this phenomenon are unknown. Parker and colleagues found that PDAC cells consumed more alanine than PSCs to fuel their

increased demand specifically through concentrative influx by the sodium-dependent neutral amino acid transporter SLC38A2. Also, multiple passive transporters, including the sodium-dependent amino acid transporter ASCT1 (encoded by *SLC1A4*), were found to be responsible for alanine secretion by PSCs. In the absence of SLC38A2, PDAC cells underwent a compartmentalized metabolic and proliferative crisis characterized specifically by alanine uptake deficiency, despite the fact that SLC38A2 also transports other amino acids. The authors found that PDAC cells downregulated cytosolic alanine biosynthetic machinery

and depended on SLC38A2 to maintain intracellular alanine levels. SLC38A2 deficiency caused PDAC cells to transition from an anabolic to a more catabolic state, increasing utilization of glutamine and branched-chain amino acid nitrogen for *de novo* alanine biosynthesis. Ultimately, SLC38A2-deficient PDAC cells failed to restore proliferation rates after long-term culture, suggesting ineffective metabolic compensation and lack of alanine transport redundancy. Additionally, SLC38A2 was selectively overexpressed in cancer cells relative to the surrounding stroma in human and murine PDAC tumor specimens. The *in vivo* relevance of these findings was verified by subcutaneous and orthotopic experiments, which showed that SLC38A2 deficiency in injected PDAC cells caused tumor regression and a decreased tumor-initiative capacity. Together, this study demonstrates how metabolic interactions between stromal and cancer cells can be facilitated by cell-specific expression of transporters and suggests that SLC38A2 may be a viable therapeutic target to specifically inhibit alanine cross-talk between PSCs and PDAC cells. ■

See article, p. 1018.

## New Prostate Cancer Modeling Method Reveals Druggable Vulnerability

- A novel electroporation-based genetically engineered mouse model of prostate cancer was designed and validated.
- Using the model, a vulnerability to tankyrase inhibition in WNT pathway-activated prostate cancer was discovered.
- This shows the utility of a rapid and targeted method to introduce genetic modifications into the mouse prostate.



Traditional genetically engineered mouse models (GEMM) of prostate cancer are valuable, but their production is time-consuming and costly, and tumors that arise in such models occur sporadically after long periods of time. To address this, Leibold, Ruscetti, Cao, and colleagues developed electroporation-based

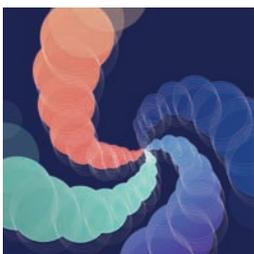
GEMMs (EPO-GEMM), which enabled rapid and targeted introduction of genetic modifications into the prostate at chosen time points. EPO-GEMMs in which a *MYC*-containing transposon vector (to model *MYC* amplification) and a plasmid expressing *sgTrp53* and *Cas9* (to edit *Trp53*) had been delivered to the prostate, termed *MP* mice, recapitulated features of *TP53*-mutant advanced human prostate cancer, such as histologic characteristics, castration resistance, genomic instability, and metastasis. Some of the *MP* tumors developed

WNT pathway activation, which was associated with more aggressive disease and metastasis, mimicking the effects of WNT pathway activation in human prostate cancer. The relationship between WNT pathway activation and metastasis appeared to be causal, as indicated by experiments with EPO-GEMMs; for example, WNT pathway activation via *Apc* mutation led to metastasis in 100% of *MP* mice versus 64% of *MP* mice without *Apc* disruption. The relationship between *Apc* mutation-driven WNT pathway activation and prostate cancer progression, invasiveness, and metastasis was also validated in an established organoid system. Finally, treatment with G007-LK, an inhibitor of the WNT pathway regulator tankyrase, decreased tumor growth, reduced metastasis, and increased life span in prostate cancers exhibiting WNT pathway activation. This work not only demonstrates the utility of EPO-GEMMs, but also uncovers a potentially targetable vulnerability in advanced prostate cancer. ■

See article, p. 1038.

## Pancreatic Cancer Escapes KRAS Dependence via Microenvironment Changes

- The histone deacetylase HDAC5 promoted oncogenic KRAS-independent pancreatic ductal adenocarcinoma (PDAC) growth.
- HDAC5 lowered *Socs3* expression to increase CCL2, attracting macrophages that provide TGF $\beta$  to sustain cancer cells.
- HDAC5 and other participants in this pathway were druggable in mice, making them targets of interest in PDAC.



Oncogenic *KRAS* mutations drive initiation and maintenance of pancreatic ductal adenocarcinoma (PDAC). Hou and colleagues performed a functional genomic screen for epigenetic-regulator genes that enable oncogenic *KRAS*-independent PDAC growth. The screen identified *HDAC5*, encoding a histone deacetylase, as a gene that allowed PDAC growth in the absence of oncogenic *KRAS*. Further investigation showed that HDAC5-promoted, oncogenic *KRAS*-independent PDAC growth was dependent on the cell-extrinsic factor TGF $\beta$  *in vitro*, in 3-D culture, and *in vivo*, and this phenomenon was mediated by the canonical SMAD3/4-activating TGF $\beta$  pathway. In mouse PDACs that persisted without oncogenic *KRAS* via an HDAC5-mediated mechanism, there was a substantial change in the myeloid-cell population in the tumor microenvironment (TME), with

neutrophils being replaced by macrophages, which were essential for tumor growth in this context. Mechanistically, HDAC5-induced PDAC persistence and macrophage recruitment in the absence of oncogenic *KRAS* depended on the chemokine CCL2, a chemoattractant for CCR2<sup>+</sup> macrophages. This occurred due to HDAC5-mediated downregulation of *Socs3*, a negative regulator of cytokine signaling. In a xenograft model of PDAC, triple combination treatment with the *KRAS*<sup>G12C</sup> inhibitor ARS-1620, the MEK inhibitor trametinib, and the HDAC4/5 inhibitor LMK-235 suppressed tumor growth more than treatment with ARS-1620 and trametinib alone. Further, in immunocompetent hosts in which oncogenic *KRAS* expression had been halted following PDAC development, adding inhibition of HDAC4/5, TGFBR1, CCR2, or CCL2 further suppressed tumor growth and increased survival. Collectively, this work shows how PDAC can alter the TME to escape dependence on oncogenic *KRAS*, providing potentially druggable targets. ■

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See article, p. 1058.

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