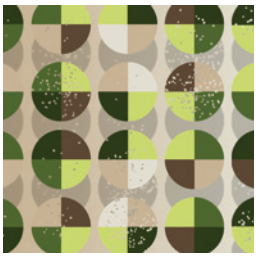
Somatic Mutations in Gastrointestinal Cancer Produce Neoantigens

- Single-nucleotide variants comprise the majority of neoantigens in gastrointestinal cancer.
- A rapid, high-throughput screen identifies patient-derived, neoantigen-reactive T cells.
- Identification of neoantigens in non-responsive cancers may increase the efficacy of immunotherapy.

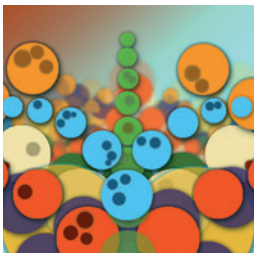


Successful immune surveillance and tumor clearance by T lymphocytes is often due to recognition of neoantigens produced from tumor-specific somatic mutations. Although this has been associated with the of success of immunotherapy against malignancies with a high mutation rate, tumors with low numbers of mutations respond poorly to immunotherapy; whether this limitation is due to inherent characteristics of the immune cells or the tumors themselves remains unclear. To identify neoantigen-reactive tumor-infiltrating lymphocytes, Parkhurst and colleagues combined whole-exome sequencing with high-throughput immunologic screening to analyze the landscape of somatic mutations that give rise to neoantigens in patients with mismatch repair–proficient gastrointestinal cancer. Among 75 patient samples, the number of observed mutations ranged from 22 to 928 (median = 114), with 94% of them representing single-nucleotide variants. Neoantigen-reactive T cells were present in 83% of patients, recognized 1.6% of all screened peptide variants, and exhibited minimal or no activity toward wild-type peptides. Neoantigen recognition was evident based on upregulation of 4-1BB or secretion of IFNγ from reactive T cells, with 46% of neoantigens recognized by $CD8^+$ T cells and 54% recognized by $CD4^+$ T cells. Neoantigen reactivity was often lost following prolonged culturing of T cells *in vitro* or following loss of CD3 expression after neoantigen exposure. These results show that even cancers with low numbers of somatic mutations elicit antitumor responses from circulating T cells. Harnessing the stimulatory potential of these neoantigens may provide a means to increase the efficacy of immunotherapy in patients with these tumors. ■

See article, p. 1022.

RNA Polymerase I Inhibition Is Feasible in Patients with Blood Cancers

- CX-5461 is active in patients with relapsed or refractory hematologic malignancies.
- CX-5461 inhibits rDNA transcription independent of *TP53* mutational status.
- CX-5461 may be effective in patients who fail to respond to standard-of-care therapy.



Inhibition of ribosome biogenesis has been proposed as a potential therapeutic strategy in multiple cancers, as cells with high rates of cellular division require significant transcription of ribosomal DNA genes (rDNA) via RNA Polymerase I (Pol I). In a phase I dose-escalation study, Khot, Brajanovski, and colleagues assessed the safety and pharmacokinetic profile of CX-5461, a small-molecule inhibitor of Pol I–mediated transcription, in patients with relapsed and refractory hematologic malignancies. The primary objective was to determine safety and tolerability of CX-5461, and secondary objectives were to assess pharmacokinetics and pharmacodynamics, preliminary antitumor activity, and the impact of *TP53* mutational status as a predictive biomarker. Sixteen patients received increasing doses of CX-5461 for up to seven cycles of therapy. The maximum tolerated dose of CX-5461 was 170 mg/m^2. Adverse events included photosensitivity, neutrophil and platelet count decreases, anemia, and palmar–plantar erythrodysesthesia, the latter of which presented as a dose-limiting toxicity. CX-5461 reached maximum plasma concentration within 60 minutes of intravenous administration, and the mean terminal half-life was 45.5 hours. Following therapy, one patient with anaplastic large-cell lymphoma exhibited a sustained partial response for more than 12 months. A fluorescence *in situ* hybridization assay developed for this study confirmed on-target drug activity against Pol I, demonstrating rDNA transcription inhibition independent of *TP53* mutational status and CX-5461 dosage. These results suggest that CX-5461 should be evaluated further as a monotherapy or in combination with established targeted therapies in hematologic cancers. ■

See article, p. 1036.

RAS Pathway Activation Drives Secondary Resistance to Gilteritinib

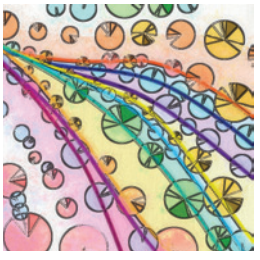
- Inhibition of FLT3 induces complex patterns of clonal selection and evolution in patients with AML.
- RAS pathway activation is the most common secondary resistance mechanism to FLT3 inhibition in AML.
- Combinatorial targeted therapies may overcome resistance to gilteritinib monotherapy in AML.

The class III receptor tyrosine kinase Fms-Like Tyrosine kinase 3 (encoded by *FLT3*) is frequently mutated in patients with acute myeloid leukemia (AML). Several second-generation FLT3 inhibitors are efficacious as monotherapies in patients with relapsed/refractory *FLT3*-mutant AML, but their efficacy is frequently curtailed by the acquisition of on-target resistance mutations in the FLT3 tyrosine kinase domain (TKD). Gilteritinib is a recently approved, type 1 FLT3 inhibitor that shows clinical activity against the recurrent internal tandem duplication (*FLT3*-ITD) mutations associated with AML relapse and *FLT3*-TKD mutations that confer secondary resistance to type II FLT3 inhibitors. McMahon and colleagues evaluated paired pre- and post-treatment samples from patients with AML who were treated with gilteritinib to identify potential secondary resistance mechanisms. The most common mechanism of gilteritinib resistance was the development of activating RAS/MAPK pathway mutations; the emergence of on-target *FLT3*^{F691L} mutations, which was the next most common gilteritinib resistance mechanism, occurred in a minority of patients and, as predicted by prior *in vitro* studies, only at relatively lower doses of gilteritinib. Further, serial single-cell targeted sequencing identified different, sometimes complex patterns of clonal evolution during FLT3 inhibition and showed that resistance mutations may arise well before relapse. These findings confirm that relapsed/refractory AML is genetically simple, yet highly polyclonal, and that the dose of gilteritinib potentially modulates mechanisms of secondary resistance to gilteritinib but not its ultimate generation. Further, the data identify potential combinatorial approaches that may enhance the efficacy of gilteritinib in patients with *FLT3*-mutant AML. ■

See article, p. 1050.

The FGFR Inhibitor TAS-120 Is Active Against FGFR2 Resistance Mutations

- TAS-120, an irreversible FGFR inhibitor, can overcome acquired resistance mutations in FGFR2.
- FGFR inhibitors exhibit distinct profiles of secondary FGFR2 resistance mutations.
- Strategic sequencing of FGFR inhibitors may prolong clinical benefit in *FGFR2* fusion–positive ICC.

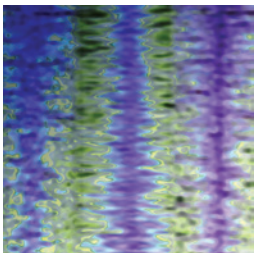


Intrahepatic cholangiocarcinoma (ICC) is an aggressive malignancy for which there are limited treatment options. Recent clinical trials have demonstrated the efficacy of ATP-competitive FGFR kinase inhibitors including BGJ398 and Debio1347; however, acquired resistance, often mediated by secondary mutations in FGFR2, prevents sustained clinical responses. Goyal and colleagues assessed the efficacy of TAS-120, an irreversible FGFR inhibitor, in four patients with *FGFR2* fusion–positive ICC whose disease initially responded and then progressed following treatment with BGJ398 or Debio1347. Analysis of tumor biopsies and circulating tumor DNA (ctDNA) revealed that progression was associated with the emergence of multiple secondary mutations in the FGFR2 kinase domain. Subsequent therapy with TAS-120 resulted in partial responses in two patients and stable disease in two patients, suggesting the ability of TAS-120 to overcome resistance to ATP-competitive FGFR inhibitors. Functional modeling in patient-derived ICC cell lines demonstrated dependence of these tumors on sustained FGFR signaling and identified distinct resistance profiles for each FGFR inhibitor. These studies also confirmed the activity of TAS-120 against several secondary FGFR2 resistance mutations, consistent with the clonal dynamics observed in analyses of ctDNA from patients. Furthermore, structural modeling supported a molecular basis for these differential drug responses, including evidence that covalent binding by TAS-120 may contribute to sustained activity against several FGFR2 mutations. These findings demonstrate the clinical utility of TAS-120 and suggest that strategic sequencing of FGFR inhibitors, guided by serial tumor biopsies and ctDNA analyses, may extend the duration of benefit in patients with *FGFR2* fusion–positive ICC. ■

See article, p. 1064.

Human HSCs Exhibit Age-Related Epigenetic Reprogramming

- Aging results in epigenetic reprogramming of DNA methylation and histone modifications in human HSCs.
- Epigenetic changes in developmental and cancer pathways are also found in patients with AML of all ages.
- Age-associated epigenetic changes may impair HSC function and predispose to myeloid malignancies.

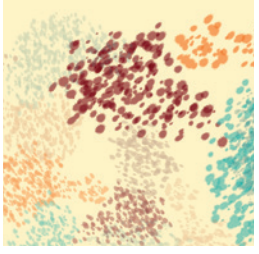


Although aging is associated with increased risk of developing myeloid malignancies, the precise events that contribute to malignant transformation of hematopoietic stem cells (HSC) remain unknown. To identify factors that contribute to this risk, Adelman and colleagues assessed age-related changes in the epigenetic and transcriptional landscape of human HSCs from young and aged healthy donors. This integrative analysis revealed that aged normal HSCs were characterized by reproducible and genome-wide reprogramming of both DNA methylation and histone modifications targeting genes involved in development and cancer. Age-associated deregulation of active enhancers was linked to genes involved in epigenetic modification, lymphoid and immune signaling, and myeloid leukemia. Loss of histone H3 lysine 4 trimethylation at bivalent promoters during aging was associated with a switch from bivalency to repression at genes involved in WNT and Hedgehog signaling as well as cancer-related pathways. Of note, several of these age-associated epigenetic alterations were also found in samples from patients with acute myeloid leukemia (AML) regardless of age group. In addition, epigenetic changes in aged HSCs were associated with differential expression of genes encoding epigenetic regulators and hematopoietic transcription factors, including downregulation of Kruppel-like factor 6 (KLF6) with age. *In vitro*, knockout of *KLF6* in normal CD34$^+$ cells increased myeloid colony formation and recapitulated aged HSC transcriptional profiles as well as enrichment for AML-associated and cancer-associated genes. Taken together, these findings suggest that age-related epigenetic reprogramming in HSCs likely impairs their ability to differentiate and may predispose to malignant myeloid transformation. ■

See article, p. 1080.

Cancer-Associated Fibroblasts Have Antigen-Presenting Features in PDAC

- Single-cell analysis of PDAC fibroblasts identified a class of CAF (apCAF) with antigen presentation features.
- apCAFs express MHC class II and CD74 but not classic costimulatory molecules.
- apCAFs may contribute to the immunosuppressive PDAC microenvironment.

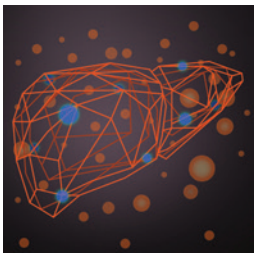


There is no effective treatment for pancreatic ductal adenocarcinoma (PDAC), and a major contributor to its progression and treatment resistance is an assortment of nonmalignant stromal cells called cancer-associated fibroblasts (CAF). Using a fibroblast-enriched single-cell transcriptomics-based approach, Elyada and colleagues profiled human PDAC tumors, adjacent normal tissue, and mouse pancreatic tumors. This technique verified that myofibroblastic CAFs (myCAF) and inflammatory CAFs (iCAF) the group had previously identified are present in PDAC tumors, enabled definition of their transcriptional profiles, and uncovered previously unidentified marker genes for these cell types. The strategy also revealed a new CAF subpopulation, dubbed antigen-presenting CAFs (apCAF), which are characterized by their expression of MHC class II–related genes, implying they may interact with CD4$^+$ T cells. Confirming this, apCAFs exhibited antigen-dependent activation of CD4$^+$ T cells *ex vivo*. Like iCAFs and myCAFs, apCAFs are dynamic, and are able to differentiate into myCAFs. Uniquely, apCAFs upregulate antigen presentation, antigen processing, fatty-acid metabolism, and MTORC1 signaling pathways as well as MYC targets. Further distinguishing apCAFs from other CAFs, apCAFs had higher activity for STAT1, which mediates MHCII expression in an IFNγ-dependent fashion. Also, apCAFs do not produce costimulatory molecules required for induction of T-cell proliferation, suggesting that the MHCII they express is a decoy receptor that deactivates CD4$^+$ T cells, adding to the immune suppression seen in PDAC, which contributes to PDAC's typical lack of response to immunotherapies. ■

See article, p. 1102.

β-Catenin Contributes to Resistance to PD-1 Inhibitors in Hepatocellular Carcinoma

- MYC;p53$^{-/-}$ murine HCCs expressing antigens are immunogenic, but β-catenin signaling is activated in immune-escaped tumors.
- β-catenin activation in MYC-driven HCCs prevents immune surveillance and causes anti–PD-1 resistance.
- *CTNNB1* mutational status may help predict which patients will respond to anti–PD-1 therapies.



The immune checkpoint inhibitors nivolumab and pembrolizumab can elicit strong responses in hepatocellular carcinoma (HCC), but only in 20% or fewer of patients, and the mechanism behind PD-1 inhibitor resistance in HCC is not known. Ruiz de Galarreta and colleagues developed a mouse model of HCC to examine the effects of genetic differences on immune surveillance, which may have implications for immunotherapy response. In the context of MYC;p53$^{-/-}$, endogenous antigen expression reduced tumor formation via T cell–mediated immune surveillance, resulting in improved survival. However, β-catenin (CTNNB1) activation in the MYC background led to immune resistance because CD103^{+} dendritic cells and antigen-specific CD8^{+} T cells that would normally restrict tumor growth are not appropriately recruited to the tumors when β-catenin is activated. *CTNNB1* is mutated in up to 37% of HCCs, and transcriptional profiling of tumors from 360 patients with HCC harboring *CTNNB1* mutations revealed reduced expression of dendritic cell markers, T-cell markers, and the exhaustion marker *PD-1*, suggesting immune exclusion. Further, an analysis of a cohort of 59 HCC patient samples showed β-catenin staining was associated with reduced CD8^{+} T-cell number. These findings suggest that *CTNNB1* mutational status may prove to be a useful biomarker for determining which patients may respond to anti–PD-1 therapies. Moreover, this mouse model of hepatocellular carcinoma may also be useful for future studies of the ways signaling pathways in tumors affect the immune system's ability to restrict their growth, potentially enabling the discovery of other mechanisms behind evasion of the immune system by cancer cells. ■

See article, p. 1124.

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