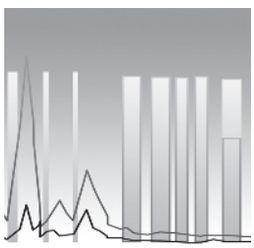
Intermittent RAF and MEK Inhibition Mitigates Paradoxical RAF Activation

- RAF inhibition in a patient with *BRAF*-mutant melanoma induced growth of an *NRAS*-mutant CMML.
- Combined use of a MEK inhibitor prevented RAF inhibitor–induced leukemic proliferation.
- An intermittent combination therapy dosing schedule led to durable responses in both cancers.

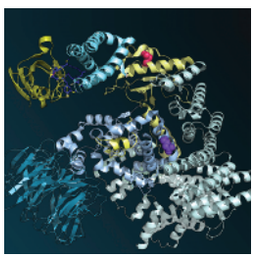


RAF inhibitors such as vemurafenib are effective in patients with *BRAF*-mutant melanoma, but paradoxically activate wild-type RAF and subsequent downstream MEK signaling in cells harboring oncogenic RAS and can thus promote the proliferation of *RAS*-mutant cancers. A previous study reported that an *NRAS*-mutant chronic myelomonocytic leukemia (CMML) was revealed in a patient with *BRAF*-mutant metastatic melanoma who was treated with vemurafenib. Because the CMML regresses quickly upon vemurafenib discontinuation, this patient has been receiving vemurafenib maintenance therapy for his melanoma on an intermittent dosing schedule. Abdel-Wahab and colleagues now report that combined treatment with vemurafenib and the MEK inhibitor cobimetinib blocked vemurafenib-induced CMML proliferation in this patient and restored his white blood cell counts to normal levels. Although drug holidays have been necessary for resolution of adverse symptoms such as fatigue, intermittent vemurafenib and cobimetinib therapy has been sufficient to maintain a near-complete melanoma response and prevent CMML progression for 85 weeks. Consistent with these findings, the level of CMML-derived *NRAS*-mutant circulating tumor DNA decreased upon combination therapy, as did the level of ERK activation in monocytes. Moreover, a comparison of the effects of RAF and MEK inhibition in myeloid leukemia cell lines revealed that inhibition of MEK, but not RAF, blocked proliferation and ERK phosphorylation in *NRAS*-mutant cells. This case report provides support for further testing of intermittent combination RAF and MEK inhibitor therapy for treatment of RAS-driven malignancies arising due to paradoxical RAF activation. ■

See article, p. 538.

An Exceptional Responder Provides Insight into Everolimus Sensitivity

- A phase I trial examined the safety of pazopanib and everolimus combination therapy.
- One patient with urothelial carcinoma had a complete radiographic response lasting 14 months.
- Whole-exome sequencing of this patient's tumor identified two activating *MTOR* mutations.



Preclinical studies have indicated that mTOR inhibitors increase the antitumor activity of antiangiogenic agents, suggesting that combining these drug classes may have clinical benefit. Wagle and colleagues conducted a phase I study to evaluate the safety, pharmacokinetics, and activity of everolimus, an allosteric inhibitor of mTOR, plus pazopanib, an inhibitor of VEGF receptors and other tyrosine kinases, in 9 patients with advanced solid tumors. The combination of everolimus and pazopanib was generally well tolerated, with toxicities consistent with everolimus or pazopanib monotherapy. However, the maximum tolerated dose of pazopanib and everolimus was 50% lower than their individual standard doses because pazopanib reduced the oral clearance of everolimus and increased the concentration of everolimus in whole blood. One patient with adrenocortical carcinoma experienced disease stabilization lasting 13 months, three patients with urothelial carcinoma had stable disease lasting 3.8 to 5.6 months, and one patient with urothelial carcinoma had a complete radiographic response that lasted 14 months. Whole-exome sequencing to identify potential mechanisms underlying this exceptional response revealed the presence of two heterozygous activating *MTOR* mutations that had not been previously reported in human cancers. The mutant mTOR proteins were highly active but remained sensitive to everolimus, providing a potential explanation for the exquisite sensitivity of this patient to treatment. In addition to identifying *MTOR* mutations as potential predictors of response to mTOR inhibitors, these findings highlight the utility of studying extraordinary responders to uncover mechanisms of drug sensitivity. ■

See article, p. 546.

Activating *MTOR* Mutations Are Associated with mTOR Inhibitor Sensitivity

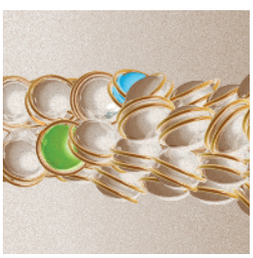
- Among mTOR pathway genes, *MTOR* has the highest percentage of recurrent mutations in human cancers.
- *MTOR* mutations cluster in distinct regions and lead to mTOR pathway activation.
- Cancer cells harboring activating *MTOR* mutations are hypersensitive to mTOR pathway inhibition.

The mTOR pathway is frequently hyperactivated in human cancers, often through mutations in upstream regulators. Mutations do occur within *MTOR*, the mTOR kinase gene, but very few cancer-associated *MTOR* mutations have been functionally characterized and the mutational spectrum of *MTOR* in human cancers remains undefined. Grabiner and colleagues catalogued mTOR pathway mutations in publicly available cancer genome datasets and observed that, although somatic point mutations occurred within almost every mTOR pathway gene, *MTOR* had the highest percentage of recurrent mutations. Over 400 samples across multiple cancer types harbored somatic nonsynonymous *MTOR* point mutations, with mutations clustering in six distinct regions of the mTOR protein. In addition to three *MTOR* mutations that had previously been shown to activate mTOR signaling, the authors identified 33 recurrent and nonrecurrent *MTOR* mutations that led to increased mTOR pathway activation, some of which showed substrate preferences consistent with differential activation of mTOR complex 1 (mTORC1) or mTORC2. A subset of these *MTOR* mutations resulted in decreased binding between mTOR and the mTOR inhibitory protein DEPTOR, suggesting a potential mechanism of mTOR activation. Although these activating mutations conferred resistance to nutrient deprivation, which may provide a growth advantage in glucose-starved developing tumors, they did not affect mTOR inhibitor sensitivity. Importantly, cancer cell lines harboring mTORC1-activating *MTOR* mutations were hypersensitive to rapamycin both *in vitro* and *in vivo*, suggesting *MTOR* mutations may confer dependence on mTOR signaling and may serve as predictive biomarkers of sensitivity to mTOR inhibitors. ■

See article, p. 554.

The NUP98–PHF23 Oncoprotein Confers Sensitivity to PHD Domain Inhibitors

- The *NUP98–PHF23* gene fusion impairs hematopoietic differentiation and induces leukemias in mice.
- NUP98–PHF23 binds a subset of H3K4me3-enriched loci and induces expression of *Hoxa* genes and *Meis1*.
- Leukemic cells with NUP98–PHD fusions are sensitive to inhibitors of PHD domain binding to H3K4me3.

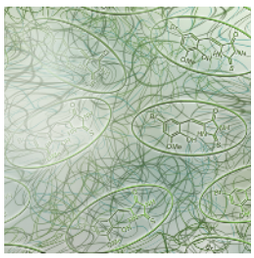


A recurrent gene fusion between nucleoporin 98kDa (*NUP98*) and plant homeodomain (PHD) finger 23 (*PHF23*) has been identified in patients with acute myeloid leukemia (AML). The NUP98–PHF23 fusion protein (NP23) retains the PHF23 PHD domain that binds trimethylated histone H3 lysine 4 (H3K4me3), suggesting that NP23 might have abnormal chromatin-modifying activity. Gough and colleagues found that transgenic mice expressing NP23 in hematopoietic tissues had significantly shorter survival compared with their wild-type littermates and developed a broad spectrum of leukemic subtypes. Hematopoietic differentiation was impaired in NP23 mice, and leukemic tissues from NP23 mice showed enrichment of a hematopoietic stem and precursor cell expression signature marked by upregulation of the *Hoxa* gene cluster and *Meis1*, similar to human AML. NP23 bound to a small subset of H3K4me3-enriched loci, and genes overexpressed in NP23 leukemias showed higher H3K4me3 occupancy, suggesting that binding and maintenance of H3K4me3 by NP23 might induce aberrant target gene expression. Interestingly, disulfiram, a clinically available compound that inhibits the interaction between PHD domains and H3K4me3, selectively killed NP23-positive AML-derived cells and decreased NP23 occupancy and transcriptional activation at target genes. Disulfiram also killed AML cells expressing a fusion between NUP98 and JARID1A, which also contains a PHD domain, but not AML cells without NUP98–PHD fusions. These results raise the possibility that compounds that disrupt PHD domain binding to H3K4me3 may have activity against NP23-driven leukemias as well as cancers driven by other PHD domain-containing proteins. ■

See article, p. 564.

c-Rel Inhibition Separates GVT Activity from GVHD

- c-Rel inhibitor-treated donor T cells reduced GVHD without affecting GVT activity.
- c-Rel deficient T cells reduce allo-activation by producing more IL2 and promoting Treg expansion.
- A small-molecule c-Rel inhibitor safely prevented GVHD and prolonged survival in mice.

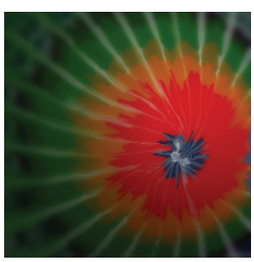


Immunosuppressant treatments for graft-versus-host disease (GVHD) arising after allogenic hematopoietic stem cell transplantation (allo-HSCT) often reduce graft-versus-tumor (GVT) activity. Given its roles in T-cell activation in alloimmunity, Shono and colleagues evaluated the NF-κB transcription factor c-Rel as a potential target for optimally modulating alloactivation and antitumor responses. c-Rel was upregulated during GVHD in mice but not required for bone marrow engraftment or reconstitution, suggesting there might be a therapeutic window for targeting c-Rel. In allo-HSCT mouse models, c-Rel–deficient donor T cells ameliorated GVHD, reduced the $CD8^+$ effector-to-regulatory T (Treg) cell ratio, and led to a decrease in the number of infiltrating donor cells in the mesenteric lymph nodes and small intestines compared with wild-type donor cells. Mechanistically, c-Rel loss relieved negative feedback on interleukin 2 (IL2) production, which resulted in greater IL2 secretion and expansion of donor-derived Treg cells. Depletion of Tregs in recipients after the transfer of c-Rel–deficient T cells reduced recipient survival, suggesting that donor-derived Tregs prevent GVHD. Pretreatment of T cells with a derivative of pyrimidinetrione, identified in a small-molecule screen as a potent, specific inhibitor of c-Rel transcriptional activity, significantly reduced GVHD following allo-HSCT while preserving GVT activity and suppressed T-cell alloreactivity in human T cells without affecting antigen-specific cytotoxicity, and systemic administration in mice following allo-HSCT safely prevented GVHD and prolonged survival. These results suggest that c-Rel inhibition may improve outcomes following allo-HSCT by separating GVHD and antitumor responses. ■

See article, p. 578.

MSH3-Mutant Cells Exhibit HR Deficiency and DNA-PKcs Addiction

- *MSH3* mutations impair HR-mediated DSB repair in cancer cells and confer dependence on DNA-PKcs.
- DNA-PKcs inhibition induces regression of established *MSH3*-mutant colorectal cancer xenografts.
- *MSH3* mutations may serve as predictive biomarkers of clinical response to DNA-PKcs inhibitors.

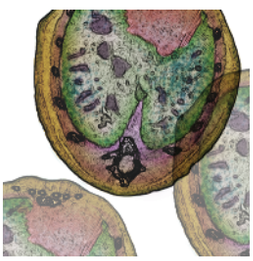


At least five different DNA repair pathways exist in human cells, with DNA double-strand break (DSB) repair specifically enacted by homologous recombination (HR) and nonhomologous end joining (NHEJ). Inactivating mutations in HR genes enhance cancer risk and confer specific genetic vulnerabilities that can be targeted therapeutically. Dietlein and colleagues set out to systematically classify genetic aberrations that are associated with nononcogene addiction to the NHEJ kinase DNA-PKcs (encoded by *PRKDC*), hypothesizing that NHEJ impairment would selectively kill HR-deficient cells. To identify genetic predictors of DNA-PKcs dependence, the authors screened a panel of 94 genomically annotated cancer cell lines for sensitivity to the DNA-PKcs inhibitor KU60648. Surprisingly, mutations in the mismatch repair pathway gene *MSH3* were found to significantly correlate with DNA-PKcs inhibitor sensitivity. Genetic or pharmacologic inhibition of DNA-PKcs caused apoptosis in *MSH3*-deficient, but not wild-type, cells, and induced regression of established *MSH3*-mutant tumors. Because mutations in HR genes were also shown to correlate with DNA-PKcs addiction, the authors hypothesized that the synthetic lethal relationship observed between *PRKDC* and *MSH3* may stem from defective HR caused by *MSH3* mutation. In support of this idea, recruitment of the HR component RAD51 to sites of DNA damage was impaired in *MSH3*-mutant cells, and inhibition of DNA-PKcs in *MSH3*-mutant cells led to the persistence of unresolved DSBs. Together, these results suggest that loss-of-function mutations of *MSH3*, which are particularly common in microsatellite-instable colorectal cancer, may be predictive of sensitivity to DNA-PKcs inhibitors currently in clinical development. ■

See article, p. 592.

NF1 Deficiency Drives EGFR Inhibitor Resistance

- A genome-wide siRNA screen identified low *NF1* expression as a resistance mechanism to EGFR inhibitors.
- EGFR inhibitors fail to inhibit RAS–MAPK signaling when *NF1* expression is reduced.
- MEK inhibitors restore the sensitivity of *NF1*-deficient mouse lung tumors to EGFR inhibition.



Lung adenocarcinomas harboring activating EGF receptor (*EGFR*) mutations initially respond to EGFR kinase inhibitors such as erlotinib, but rapidly acquire resistance. The secondary $EGFR^{T790M}$ mutation drives resistance in a majority of cases, but additional resistance mechanisms remain to be identified. In a genome-wide siRNA screen, de Bruin and colleagues found that reduced expression of neurofibromin 1 (*NF1*), a known tumor suppressor gene that encodes a RAS GTPase activating protein, conferred erlotinib resistance in an *EGFR*-mutant human lung cancer cell line. Similarly, reduced *NF1* expression was observed *in vivo* in erlotinib-resistant $EGFR^{T790M}$-negative lung tumors, suggesting that NF1 loss can drive erlotinib resistance independently of known resistance mechanisms. Erlotinib failed to fully inhibit RAS–MAPK signaling when NF1 was depleted, but combined erlotinib and MEK inhibition completely abolished this residual MAPK activation, restored erlotinib sensitivity *in vitro*, and reduced tumor growth of NF1-deficient mouse xenografts. Combined erlotinib and MEK inhibition also induced significant regression of a majority of established erlotinib-resistant tumors in a mouse model of *EGFR*-driven lung cancer. Notably, reduced *NF1* expression was detected in a significant fraction of human lung adenocarcinomas with acquired erlotinib resistance, and low *NF1* levels in pretreatment samples strongly correlated with decreased overall survival following erlotinib therapy. These findings point to a role of NF1 in EGFR inhibitor sensitivity and support further clinical evaluation of concomitant use of EGFR and MEK inhibitors in patients with refractory $EGFR^{T790M}$-negative lung adenocarcinomas. ■

See article, p. 606.

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