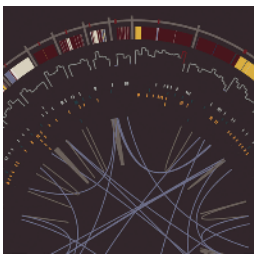
RAD50 Mutation Inhibits ATM and Sensitizes Cancer Cells to Chemotherapy

- Whole-genome sequencing identified a hypomorphic *RAD50* mutation in an outlier responder patient.
- Tumor-specific *RAD50* mutations impair ATM activation and confer dependency on ATR signaling.
- ATM-deficient tumors may benefit from combined checkpoint inhibition and genotoxic chemotherapy.



Despite recent advances in targeted therapy, curative response in metastatic solid tumors remains a challenge due to inherent or acquired resistance and disease progression. In an effort to define the underlying mechanism responsible for the durable complete response achieved in a patient with metastatic small-cell ureter cancer treated with the checkpoint kinase 1/2 inhibitor AZD7762 and the topoisomerase I inhibitor irinotecan, Al-Ahmadie, Iyer, Hohl, and colleagues performed whole-genome sequencing of tumor and matched normal tissue. This analysis revealed a complex genetic landscape characterized by *TP53* mutation and frequent DNA copy-number alterations. Integration of this data with mechanisms of drug action highlighted *RAD50*^{L1237F} as a potential sensitizing mutation. Indeed, the clonal *RAD50*^{L1237F} allele was accompanied by LOH of the wild-type allele and mutated a highly conserved residue within the D-loop structure of RAD50, which is required for proper MRE11 complex function in DNA repair. Generation of yeast strains harboring *rad50* D-loop mutations or other tumor-associated *rad50* mutations revealed that despite intact MRE11 complex formation, *rad50*-mutant cells were unable to activate ataxia telangiectasia mutated (ATM) signaling, leading to a synthetic lethal effect when DNA damage checkpoint inhibition via ataxia telangiectasia and Rad3-related (ATR) suppression was combined with DNA-damaging cytotoxic chemotherapy. Together, these findings highlight the utility of whole-genome sequencing of extreme outlier responders in dissecting tumor-specific dependencies and provide a rationale for combining checkpoint inhibitors with DNA-damaging chemotherapy in patients whose tumors harbor MRE11 complex mutations. ■

See article, p. 1014.

CDK4 Combination Therapy Overcomes Ibrutinib Resistance in MCL

- The *BTK*^{C481S} mutation is specific to relapse following durable ibrutinib responses in MCL.
- BTK^{C481S} increases AKT activation and CDK4-driven, tissue-specific proliferation in the spleen.
- CDK4 blockade improves ibrutinib efficacy in *BTK*^{WT} cells and PI3K inhibition in *BTK*^{WT} and *BTK*^{C481S} cells.

Treatment of relapsed/refractory mantle cell lymphoma (MCL) with the Bruton's Tyrosine Kinase (BTK) inhibitor ibrutinib results in unprecedented clinical responses, but long-term efficacy is limited by the development of resistance. To identify mechanisms of acquired ibrutinib resistance, Chiron, Di Liberto, Martin, and colleagues performed longitudinal integrative whole-exome and whole-transcriptome sequencing of serial tumor biopsies from ibrutinib-treated patients with MCL. This analysis identified a relapse-specific mutation also detected in chronic lymphocytic leukemia, *BTK*^{C481S}, in patients with acquired resistance following a durable ibrutinib response, but not in patients with primary ibrutinib resistance or those with acquired resistance following a brief response. Increased AKT activation was associated with the *BTK*^{C481S} mutation, but was also found in patients with primary resistance and BTK wild-type (*BTK*^{WT}) cells. *BTK*^{C481S}-mutant MCL cells preferentially proliferated in the spleen at relapse due to activation of cyclin-dependent kinase 4 (CDK4) and accelerated progression through the G1 phase. In ibrutinib-resistant *BTK*^{WT} MCL cells, induction of prolonged early G1 arrest (pG1) via CDK4 inhibition improved sensitivity to ibrutinib; this effect was dependent on synergistic induction of the PI3K inhibitor PIK3IP1 and inactivation of BTK, AKT, and classical NF-κB signaling in response to induction of pG1 and ibrutinib. Furthermore, induction of pG1 also sensitized both *BTK*^{WT} and *BTK*^{C481S}-mutant MCL cells to killing by PI3K inhibitors. These findings provide insight into the mechanisms of resistance in ibrutinib-treated MCL and indicate potential therapeutic strategies to overcome ibrutinib resistance. ■

See article, p. 1022.

Afatinib Plus Cetuximab Is Active in EGFR TKI–Resistant *EGFR*-Mutant NSCLC

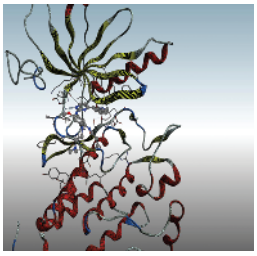
- The combination of afatinib and cetuximab in *EGFR*-mutant NSCLC was assessed in a phase Ib trial.
- Heavily pretreated patients with acquired resistance to erlotinib/gefitinib were enrolled.
- The combination induced durable responses independent of T790M status and was well tolerated.

Despite initial clinical responses, most patients develop acquired resistance to the first-generation EGFR tyrosine kinase inhibitors (TKI) erlotinib and gefitinib, often via the secondary *EGFR*^{T790M} mutation, underscoring the need for combinatorial therapeutic strategies. Preclinical studies suggest that dual treatment with the irreversible ERBB family inhibitor afatinib and the EGFR-targeting monoclonal antibody cetuximab can overcome erlotinib resistance in *EGFR*^{T790M}-mutant lung cancer, prompting Janjigian and colleagues to evaluate the safety and efficacy of this combination in a phase Ib clinical trial. One hundred twenty-six patients with *EGFR*-mutant lung cancer and acquired resistance to erlotinib or gefitinib received the MTD of afatinib plus cetuximab; in addition to EGFR TKIs, the majority of these patients had been heavily pretreated with cytotoxic chemotherapy. Dual treatment with afatinib and cetuximab induced an objective response (OR) rate of 29% and resulted in a median duration of OR of 5.7 months and a median progression-free survival of 4.7 months. Of note, the OR rate was similar among patients with the *EGFR*^{T790M} mutation and those without this mutation. Grade 3 and 4 treatment-related adverse events, most commonly including rash and diarrhea, were observed in 44% and 2% of patients, respectively, and dose reduction and therapy discontinuation were not required in most patients, suggesting that this combination shows a manageable safety profile. These results provide clinical evidence that EGFR TKI–resistant tumors remain dependent on EGFR signaling for survival and demonstrate that dual EGFR inhibition produces durable antitumor responses. ■

See article, p. 1036.

AZD9291 Overcomes EGFR TKI Resistance in Non–Small Cell Lung Cancer

- AZD9291 selectively inhibits drug-sensitive and EGFR^{T790M} mutants but not wild-type EGFR.
- AZD9291 induces profound, sustained tumor regression in *EGFR*-mutant mouse models of NSCLC.
- Partial responses were seen in patients with advanced NSCLC and acquired EGFR TKI resistance.

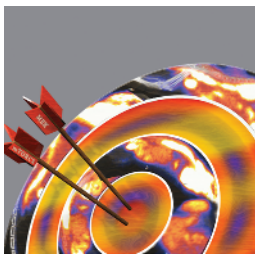


Patients with *EGFR*-mutant non–small cell lung cancer (NSCLC) initially respond to first-generation EGFR tyrosine kinase inhibitors (TKI) such as erlotinib and gefitinib, but eventually develop acquired resistance, most frequently via a second *EGFR* mutation, T790M. In addition, the efficacy of first- and second-generation EGFR TKIs is reduced by dose-limiting toxicity due to inhibition of wild-type EGFR. Cross and colleagues characterized AZD9291, an oral, irreversible selective inhibitor of mutant EGFR that covalently targets cysteine 797 but has a distinct chemical structure compared with other third-generation EGFR TKIs. AZD9291 potently suppressed EGFR phosphorylation and cell proliferation in tumor cell lines harboring drug-sensitizing EGFR mutants or the T790M resistance mutant, but not those expressing wild-type EGFR. In addition, low-dose AZD9291 demonstrated on-target inhibition of mutant EGFR *in vivo*, induced profound and durable tumor shrinkage in *EGFR*-mutant xenograft and transgenic mouse models of NSCLC including those expressing T790M, and was well tolerated, consistent with its selectivity profile. Furthermore, in an ongoing first-in-human phase I clinical trial, two patients with advanced T790M-positive NSCLC who had experienced disease progression on prior therapy with gefitinib exhibited confirmed partial radiographic responses to AZD9291, with significant tumor shrinkage and prolonged clinical benefit. Together, these results demonstrate that AZD9291 induces promising antitumor activity and support further clinical investigation of AZD9291 for the treatment of *EGFR*-mutant NSCLC, in particular T790M-driven refractory disease. ■

See article, p. 1046.

mTORC1 and MEK Are Therapeutic Targets in *NF1*-Mutant Tumors

- mTORC1 is the primary PI3K effector in *NF1*-mutant MPNST cells but mTORC2 and AKT are dispensable.
- Combined and sustained mTORC1–MEK inhibition leads to tumor regression in an MPNST mouse model.
- Reduced GLUT1-mediated ^{18}F-FDG uptake requires and is a biomarker of dual mTORC–MEK inhibition.

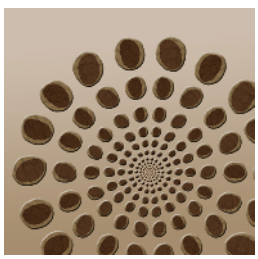


Neurofibromin 1 (*NF1*)–mutant malignant peripheral nerve sheath tumors (MPNST) are reliant on RAS pathway signaling and are resistant to conventional therapeutic agents, underscoring the need for targeted therapies. Through genetic ablation and pharmacologic inhibition of specific PI3K isoforms, Malone, Fromm, and colleagues found p110α to be the primary isoform responsible for human MPNST cell proliferation *in vitro*. In addition, whereas MPNSTs were sensitive to loss of mTOR complex 1 (mTORC1), mTORC2 and AKT were dispensable. In a genetically engineered *Nf1*-mutant MPNST mouse model, rapamycin effectively suppressed tumor growth, but the effects were cytostatic. However, the combination of rapamycin with a MEK inhibitor induced tumor regression, which required sustained target inhibition. Transcriptional profiling and protein analyses of murine MPNST tumors posttreatment identified solute carrier family 2, member 1 (*Slc2a1*), which encodes the glucose transporter GLUT1, as a gene uniquely repressed by dual mTORC1 and MEK inhibition. Consistent with these findings, GLUT1-mediated uptake of ^{18}F-fluorodeoxyglucose (^{18}F-FDG), as measured by PET scan, was significantly impaired only in mice treated with the rapamycin–MEK inhibitor combination. Furthermore, suppression of ^{18}F-FDG uptake at early time points correlated with tumor regression at 10 days post-treatment. These results indicate that mTORC1 and MEK are the principal therapeutic targets in *NF1*-mutant nervous system malignancies and highlight ^{18}F-FDG uptake as a potential noninvasive biomarker to assess the efficacy of this combination therapy in clinical trials. ■

See article, p. 1062.

Dependence on Antiapoptotic Proteins Is Linked to Differentiation State

- Most T-ALLs are dependent on BCL-XL, but the early T-cell progenitor subtype is dependent on BCL-2.
- Expression of and dependence on antiapoptotic proteins changes during T-cell maturation.
- Early T-cell progenitor ALL is sensitive to BCL-2 inhibition with the BH3 mimetic ABT-199.



Some hematologic cancers are dependent on antiapoptotic BCL-2 family proteins for survival, and BH3 domain mimetics that inhibit the function of these proteins have shown activity in preclinical studies. Using BH3 profiling, an assay that measures the ability of BH3 peptides that selectively bind and inhibit specific antiapoptotic BCL-2 family members to induce loss of mitochondrial membrane potential, Ni Chonghaile and colleagues evaluated antiapoptotic protein dependencies in T-cell acute lymphoblastic leukemia (T-ALL). The vast majority of T-ALL cell lines and primary samples were dependent on BCL-XL, with the exception of cell lines and primary samples representing the early T-cell progenitor (ETP) T-ALL subtype, which were selectively dependent on BCL-2. Analysis of BCL-2 and BCL-XL expression during thymocyte development showed that BCL-2 is highly expressed at the early progenitor stage but declines during T-cell maturation as BCL-XL expression increases, providing a potential explanation for these findings. Consistent with these results, typical T-ALL samples were more sensitive to ABT-263 (a BCL-2, BCL-XL, and BCL-W inhibitor) than to ABT-199 (a better tolerated BCL-2–specific inhibitor that spares BCL-XL–dependent platelets), whereas ETP T-ALL samples were sensitive to both BH3 mimetics, with ABT-199 eliminating ETP T-ALL cells *in vivo*. In addition to demonstrating that BCL-2 family member dependence can be determined by the cellular differentiation state, these findings identify BCL-2 as a potential therapeutic target in ETP T-ALL and suggest that ABT-199 may be an effective therapy for this treatment-resistant ALL subtype. ■

See article, p. 1074.

Mutations in Hematopoietic Progenitors Disrupt BCR Signaling and Drive CLL

- Acquired mutations are present in multipotent hematopoietic progenitors of patients with CLL.
- Genes including *BRAF*, *NFKBIE*, and *EGR2* are recurrently mutated in preleukemic progenitor cells.
- Early mutations in progenitor cells deregulate BCR signaling and impair B-cell differentiation.

Chronic lymphocytic leukemia (CLL) is characterized by clonal expansion of mature B-lymphocytes; however, it remains unclear whether CLL arises from transformation of mature B cells or from acquisition of driver mutations in hematopoietic progenitor cells. To address this question, Damm, Mylonas, and colleagues combined whole-exome and targeted sequencing of immature hematopoietic progenitor cells and mature B-cells, T-cells, and monocytes isolated from patients with CLL. Intriguingly, in the majority of patients, CLL-associated somatic mutations were detected in $CD34^+$ hematopoietic progenitor cells with multipotent differentiation potential or in $CD14^+$ myeloid cells, suggesting that mutation of immature preleukemic cells is an early event that drives CLL pathogenesis. Consistent with this idea, mutations in known CLL oncogenes, such as *NOTCH1*, *SF3B1*, and *BRAF*, were identified in progenitor cells. In addition, recurrent mutations in *NFKBIE* and *EGR2* were detected in 10.7% and 8.3% of patients, respectively, and *EGR2* mutations were associated with reduced overall survival and shorter time to treatment. Expression of mutant BRAF in hematopoietic progenitor cells affected normal B-cell maturation, suggesting that early CLL mutations converge to deregulate B-cell receptor (BCR) signaling. Indeed, *EGR2* mutation altered the expression of EGR2 target genes, resulting in a gene signature that was enriched in BCR-stimulated genes. These findings indicate that CLL arises from multipotent hematopoietic progenitor cells harboring mutations that result in aberrant B-cell differentiation. ■

See article, p. 1088.

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