Rapid Drug Development Facilitates Next-Generation TRK Inhibitor Use

- LOXO-195 overcomes acquired resistance to the first-generation TRK TKI larotrectinib.
- Parallel development allowed rapid next-generation TKI use in patients with larotrectinib resistance.
- Accelerated drug development may overcome clinical trial challenges to target rare mutations.

In drug development for rare molecular targets, the limited number of patients can prevent sequential testing of first- and second-generation inhibitors. The TRK kinases represent one such target, and *NTRK* gene rearrangements can be targeted by the recently developed selective TRK tyrosine kinase inhibitor (TKI) larotrectinib. To combat acquired resistance, Drilon and colleagues developed LOXO-195, a TRK TKI designed to overcome resistance conferred by secondary TRK kinase domain mutations, in parallel with the early clinical development of larotrectinib. *In vitro*, LOXO-195 potently inhibited wild-type NTRK1/2/3 and several TRK kinase domain mutants that reduced larotrectinib sensitivity. Further, LOXO-195 suppressed the growth of tumors driven by oncogenic NTRK1/3 in mice. Based on these preclinical findings, the first two patients to develop larotrectinib resistance were treated with LOXO-195 using rapid safety- and pharmacokinetic-guided intrapatient dose-escalation study designs. An adult patient with *LMNA–NTRK1*-rearranged colorectal cancer who developed larotrectinib resistance due to an NTRK1 G595R acquired resistance mutation responded rapidly to LOXO-195 and achieved an ongoing partial response. After treatment the G595R allele was no longer detectable in cell-free DNA. A pediatric patient with *ETV6–NTRK3*-rearranged infantile fibrosarcoma with a G623R acquired resistance mutation also experienced rapid tumor regression, though her cancer recurred. In addition to demonstrating that LOXO-195 may overcome resistance to larotrectinib in patients with *NTRK*-rearranged tumors, this study illustrates the utility of a drug development approach that anticipates resistance to first-generation kinase inhibitors and enables parallel generation of next-generation kinase inhibitors, particularly in a rare patient population. ■

See article, p. 963.

Loss-of-Function *ERF* Mutations Promote Prostate Cancer

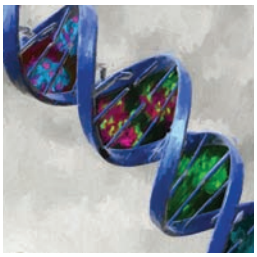
- Exome analysis of an African-American cohort shows *ERF* is mutated and deleted in prostate cancer.
- *ERF* loss increases growth and upregulates genes associated with ETS activation and androgen signaling.
- Diverse study populations may improve the identification of potential cancer drivers.

African-American men have the highest incidence rate of prostate cancer of all racial/ethnic groups in the world, which is thought to be driven by genetic differences, potential risk factors such as diet and lifestyle, and access to healthcare. To identify the genomic basis underlying the increased morbidity and mortality of African-American men with prostate cancer, Huang and colleagues genomically characterized hormone-naïve primary tumors from African-Americans with prostate cancer. Whole-exome sequencing (WES) and targeted sequencing of a discovery set of 102 matched tumor–normal pairs and a validation set of 90 matched tumor–normal pairs, respectively, identified recurrent mutations in *SPOP* and *FOXA1*, which are known to be drivers of prostate cancer, and the ETS transcriptional repressor *ERF* (5%). Further, the nonsynonomous *ERF* mutations were associated with loss of *ERF* expression, and copy-number analysis identified hemizygous loss of *ERF* in 3 (3%) patients. Similarly, analysis of other cohorts including The Cancer Genome Atlas prostate cancer cohort showed that ~3% of prostate tumors exhibited loss of *ERF* due to mutations (<1%) and deletions (2%). Expression profiling revealed that *ERF* knockdown resulted in the upregulation of androgen signaling genes, and ablation of *ERF* increased prostate cancer cell anchorage-independent and androgen-dependent growth and invasion *in vitro* and xenograft growth *in vivo*. These findings identify *ERF* as a potential tumor suppressor in prostate cancer and demonstrate the role of increasing the racial and ethnic diversity of sequencing cohorts in the identification of tumor drivers. ■

See article, p. 973.

Reversion Mutations in HR Genes Promote PARPi Resistance

- Secondary reversion mutations in *BRCA1*, *RAD51C*, or *RAD51D* can confer resistance to PARPi.
- The reversion mutations restore the open reading frame to reestablish HR activity.
- Primary mutations in *RAD51C* and *RAD51D* may confer sensitivity to PARPi in patients with cancer.

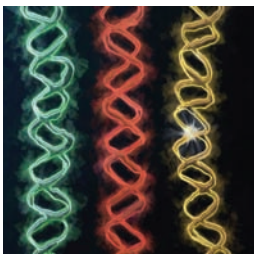


Mutations in homologous recombination (HR) genes including *BRCA1/2*, *RAD51C*, or *RAD51D* confer sensitivity to PARP inhibitors (PARPi). To identify PARPi resistance mechanisms, Kondrashova and colleagues performed targeted sequencing of 12 paired pretreatment and postprogression biopsies from patients with high-grade epithelial ovarian carcinoma treated with the PARPi rucaparib. In 6 of 12 patients, mutations that disrupted HR were detected: *BRCA1* in 4 patients, *RAD51C* in 1 patient, and *RAD51D* in 1 patient. Each of these patients initially derived clinical benefit from rucaparib, and after progression 5 of the 6 patients harbored secondary mutations that restored the open reading frame of the mutant HR gene. In the *BRCA1*-mutated cases the secondary mutations were large in-frame deletions that either deleted the primary frameshift mutation or shifted the reading frame back to the correct position. In the case with a germline *RAD51C* mutation, 4 secondary mutations were detected that restored the open reading frame and HR activity. In the patient with a germline *RAD51D* mutation, postprogression biopsies were collected from a liver metastasis that was still responding to treatment and a spleen metastasis that was not. The secondary *RAD51D* mutation was observed only in the splenic lesion, suggesting that it contributed to PARPi resistance. Further, the *RAD51C/D* reversion mutations conferred resistance to multiple PARPi *in vitro*. These findings indicate that mutations in *RAD51C* and *RAD51D* are synthetically lethal with PARPi and that secondary reversion mutations in *BRCA1*, *RAD51C* and *RAD51D* can confer resistance to PARPi in patients with HR-deficient tumors. ■

See article, p. 984.

BRCA2 Reversion Mutations Promote Resistance to PARPi

- cfDNA copy-number analysis detected *BRCA2* reversion mutations in two patients with prostate cancer.
- The reversion mutations are associated with resistance to the PARPi olaparib and talazoparib.
- cfDNA allows for monitoring of PARPi resistance in patients with prostate cancer.

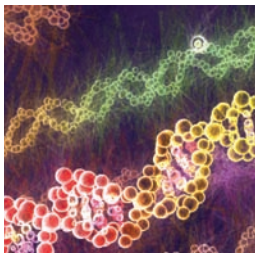


Approximately 20% of metastatic prostate cancers harbor inactivating mutations in genes required for DNA repair (such as *BRCA2*), most frequently affecting double-strand DNA repair by homologous recombination (HR). These tumors are sensitive to PARP inhibitors (PARPi) including olaparib and talazoparib. In ovarian and breast cancers, olaparib resistance has been associated with somatic reversion mutations that reactivate nonfunctional HR-associated genes. Quigley, Alumkal, and colleagues analyzed DNA from solid tumor biopsies and circulating cell-free DNA (cfDNA) and demonstrated that patients with *BRCA2*-mutant metastatic prostate cancer treated with talazoparib or olaparib develop reversion mutations coincident with the occurrence of resistance. Patient 1 bore a pathogenic germline single-nucleotide *BRCA2* variant producing a premature stop codon. DNA sequencing of solid tumor biopsies obtained before talazoparib treatment and after resistance identified two reversion deletions in *BRCA2* that eliminated the stop codon and restored the open reading frame in talazoparib-resistant samples. Analysis of cfDNA obtained after resistance identified 5 additional reversion alleles not found in the solid tumor biopsy. Patient 2, treated with olaparib, bore a pathogenic germline two nucleotide deletion in *BRCA2*, and cfDNA analysis identified 34 distinct alleles with indel mutations predicted to restore the open reading frame of *BRCA2*. Collectively, these findings demonstrate the use of cfDNA to identify heterogeneous reversion mutations as a mechanism of resistance to PARPi in patients with metastatic prostate cancer. ■

See article, p. 999.

cfDNA Analysis Predicts Response to Olaparib in Prostate Cancer

- Reduced cfDNA levels are linked to extended survival in patients with olaparib-treated prostate cancer.
- cfDNA sequencing identifies resistance mutations including reversion mutations in *BRCA2* and *PALB2*.
- cfDNA analysis may predict response and resistance in patients with prostate cancer to guide therapy.

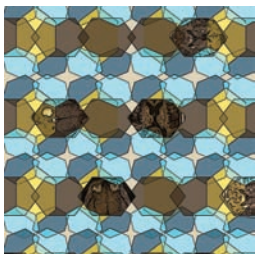


The PARP inhibitor olaparib showed antitumor activity in patients with metastatic prostate cancer in the phase II TOPARP-A trial. Serial circulating cell-free DNA (cfDNA) samples were collected during this trial, and Goodall, Mateo, and colleagues performed targeted and whole-exome sequencing to determine if changes in cfDNA concentration or mutations detected in cfDNA are predictive of response and resistance. Overall, 16 of 49 evaluable patients responded to olaparib, 14 of whom had defects in homologous recombination (HR) DNA repair, and serial cfDNA samples were available from 46 patients. A reduction in cfDNA concentration after olaparib treatment was associated with extended progression-free and overall survival. Targeted next-generation sequencing detected somatic mutations in the majority of baseline cfDNA samples, and the mutant allele frequency decreased with olaparib treatment in responding patients, but not in nonresponding patients. Six patients in the TOPARP-A trial had somatic tumor mutations in genes associated with HR deficiency, 5 of whom responded to olaparib, and these mutations were detectable in baseline cfDNA and decreased in frequency in responding patients. Sequencing of paired samples collected from 7 patients before olaparib treatment and at disease progression revealed mechanisms of resistance to olaparib. In patients with germline or somatic frameshift mutations in *BRCA2* or *PALB2*, additional somatic mutations occurred that restored the open reading frame. Altogether, these findings suggest that cfDNA analysis can be used to predict response and resistance in patients with metastatic prostate cancer and may guide patient care. ■

See article, p. 1006.

Primary CNS Lymphoma Is Responsive to BTK Inhibition

- The BTK inhibitor ibrutinib is active and safe in patients with relapsed or refractory CNS lymphoma.
- Mutations in *CD79B* lead to incomplete ibrutinib response via activation of PI3K/mTOR signaling.
- Inhibition of PI3Kα/δ or mTOR synergizes with ibrutinib to induce cell death in *CD79B*-mutant tumors.

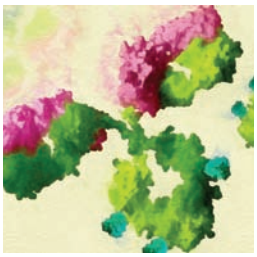


Ibrutinib, a first-in-class, oral inhibitor of Bruton tyrosine kinase (BTK), has demonstrated clinical activity in patients with B-cell malignancies, including diffuse large B-cell lymphoma (DLBCL). However, it is not known whether BTK represents a viable therapeutic target in DLBCL tumors of the central nervous system (CNS), which include primary CNS lymphomas (PCNSL) and relapsed secondary CNS lymphomas (SCNSL). Grommes, Pastore, Palaskas, and colleagues conducted an open-label, nonrandomized, dose-escalation phase I trial to evaluate the tolerability and activity of single-agent ibrutinib in twenty patients with relapsed or refractory PCNSL or SCNSL. Ibrutinib exhibited a manageable safety profile and higher response rates compared with DLBCL outside the CNS. Clinical responses were observed in 10 (77%) of 13 patients with PCNSL, including five complete responses (CR) and five partial responses, and in 5 (71%) of 7 patients with SCNSL, including 4 CRs. The median progression-free survival was 4.6 months among patients with PCNSL and 7.4 months among patients with SCNSL. Genomic analyses of PCNSL biopsies identified mutation of caspase recruitment domain family member 11 (*CARD11*), a known resistance mechanism, in a patient with complete ibrutinib resistance. In addition, mutations in the B-cell receptor–associated protein CD79B activated BTK-independent PI3K/mTOR prosurvival signaling and were associated with incomplete ibrutinib responses. Combined treatment with ibrutinib and inhibitors of the PI3K isoforms p110α/p110δ or mTOR synergistically induced PCNSL cell death in preclinical disease models. These findings demonstrate the BTK dependency of PCNSL and support further assessment of ibrutinib combination therapies for CNS lymphomas. ■

See article, p. 1018.

Cadherin-6 Is a Therapeutic Target for Ovarian and Renal Cancers

- CDH6 is overexpressed in ovarian and renal tumors and is targetable by antibody–drug conjugates.
- The antibody-drug conjugate HKT288 induced CDH6-dependent regression in an ovarian PDX clinical trial.
- HKT288 may be a potential therapy for patients with CDH6-expressing tumors.



There are limited treatment options for patients with ovarian or renal cancer, and the prognosis for these patients remains poor. To assess the potential of antibody–drug conjugates as a therapeutic approach for these patients, Bialucha and colleagues interrogated The Cancer Genome Atlas ovarian and renal cancer and the Gene-Tissue Expression normal tissue datasets and identified cadherin-6 (*CDH6*) as a top candidate for biotherapeutic targeting. A multipronged antibody generation approach identified 38 anti-CDH6 IgGs comprising a range of affinities, epitopes, and cellular potency in a surrogate antibody–drug conjugate cytotoxicity assay *in vitro*. *In vivo* screening of a diverse subset of 10 IgGs as antibody–drug conjugates using the SMCC-DM1 linker/payload identified LTV977 as the lead anti-CDH6 antibody. Additional *in vivo* profiling revealed that LTV977 conjugated to a cleavable sulfonate-bearing linker/payload (sulfo-SPDB-DM4) induced significant regression of ovarian cell line and patient-derived (PDX) xenografts, including a PDX resistant to standard-of-care therapy, compared to LTV977 conjugated to SPDB-DM4 or a non-cleavable linker/payload (SMCC-DM1). Further, the antibody–drug conjugate CDH6–sulfo-SPDB-DM4 (termed HKT288) exhibited an acceptable toxicity profile in rats and nonhuman primates and was efficacious against CDH6-expressing renal cancer xenografts *in vivo*. In an ovarian PDX clinical trial, HKT288 promoted the CDH6-dependent tumor regression of 12 (40%) of 30 ovarian PDX. These findings identify CDH6 as a therapeutic target for patients with ovarian and renal cancers and describe an integrated pharmacologic approach for designing efficacious antibody–drug conjugates. ■

See article, p. 1030.

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