Biomarker profiling for therapy selection in gastroesophageal adenocarcinoma is typically performed on endoscopic biopsies from a single primary tumor site, which assumes that the primary tumor and metastases are genomically homogeneous. Pectasides, Stachler, Derks, and colleagues hypothesized that baseline heterogeneity between primary and metastatic lesions may explain why biomarker-guided therapies have been relatively ineffective in gastroesophageal adenocarcinoma. Whole-exome sequencing of paired synchronous primary and metastatic tumors revealed that an average of 42% of point mutations and insertions/deletions and 63% of amplified genes were discordant, and clinically relevant discrepancies were identified in 45% of patients. Multiregion sequencing of primary tumors revealed similar heterogeneity and discordance with matched metastases. To demonstrate the potential relevance of these findings for patient management, the authors present observations from the ongoing Personalized Antibodies for GastroEsophageal Adenocarcinoma (PANGEA) trial, in which the primary tumor, at least one metastasis, and circulating cell-free DNA (cfDNA) were profiled for each patient. Although the primary and metastatic samples were discordant in a third of patients, targetable gene amplifications identified in cfDNA were highly concordant (>85%) with those in metastatic lesions. Treatment reassignment based on the biomarker status of the metastasis and cfDNA led to reductions in tumor burden and sustained clinical benefit in patients with genomically discordant primary and metastatic samples. These findings suggest that profiling of a single primary gastroesophageal adenocarcinoma site may lead to suboptimal treatment decisions due to genomic heterogeneity and that profiling of metastases or cfDNA may guide more effective use of targeted therapy in this disease.

See article, p. 37.

Genomic Profiling Identifies Predictive Biomarkers in Esophagogastric Cancer

- Prospective, targeted sequencing was performed on 295 patients with stage IV esophagogastric cancer.
- Patients with HER2 amplification or microsatellite instability could be identified by NGS.
- Genomic profiling may identify patients most likely to benefit from trastuzumab or immunotherapy.

Responses to standard therapies in esophagogastric cancer (platinum-based chemotherapy, trastuzumab, or anti-PD-1 antibodies) are not durable, and less than 5% of patients with metastatic disease survive longer than 5 years. Janjigian and colleagues sought to identify predictive biomarkers of response to metastatic esophagogastric cancer therapy through prospective targeted next-generation sequencing (NGS) of paired tumor and normal samples from 295 patients. Of 187 patients with HER2+ tumors treated with platinum-based chemotherapy, homologous recombination deficiency was not predictive of progression-free survival (PFS). Among 50 patients classified as HER2+ by standard assays who received first-line trastuzumab, patients with the highest levels of HER2 amplification had significantly longer PFS, whereas patients with co-occurring alterations in RTK–RAS–PI3K/AKT pathway genes had shorter PFS. Four patients not found to harbor HER2 amplification based on NGS had significantly shorter PFS, suggesting that NGS-based detection of HER2 amplification might more effectively guide trastuzumab use. Analysis of post-treatment samples suggested that loss of HER2 amplification or acquisition of HER2 exon 16 deletion or oncogenic KRAS or PIK3CA mutations may contribute to trastuzumab resistance. The 9 patients in the cohort with high microsatellite instability (MSI+) rapidly progressed on first-line platinum-based chemotherapy, indicating that immune checkpoint blockade should be considered as soon as possible for these patients. Of 5 MSI+ patients treated with immune checkpoint inhibitors, 2 had responses lasting longer than 6 months. These results demonstrate the feasibility of prospectively performing targeted NGS in metastatic esophagogastric cancer to identify biomarkers of drug response and its potential utility in optimizing treatment choices for this lethal disease.

See article, p. 49.
Inhibitors of receptor tyrosine kinases or their downstream effector kinases can lead to initial clinical responses in patients with particular genomic alterations, but these targeted therapies quickly induce adaptive transcriptional responses that facilitate the persistence and proliferation of a drug-tolerant cell population in which a wide range of genetic and nongenetic resistance mechanisms ultimately promote outgrowth of resistant tumors. Rusan and colleagues hypothesized that inhibition of cyclin-dependent kinase 7 (CDK7) and CDK12, which regulate RNA polymerase II–dependent transcription, would block adaptive transcriptional responses to targeted therapy and provide a broadly effective approach to prevent the emergence of drug-resistant cell populations. Indeed, use of a recently developed covalent CDK7/CDK12 inhibitor, THZ1, suppressed the development of acquired resistance to a panel of targeted therapies in vitro across cellular models with diverse oncogenic dependencies and lineages and was more broadly effective than established combination therapies at preventing outgrowth of resistant cells. Combining THZ1 with FGFR-, EGFR-, or ALK-targeted therapies in mouse xenograft and genetically engineered models was also well tolerated and led to significantly decreased tumor growth and increased survival compared with monotherapy. Moreover, THZ1 treatment blocked the induction of prosurvival transcriptional programs necessary for adaptive responses. Overall, these findings suggest that targeting transcription through CDK7/CDK12 inhibition may be a broadly effective approach to prevent the emergence of drug-tolerant cell populations and enhance the efficacy of targeted therapies without needing to anticipate or inhibit specific resistance mechanisms.

See article, p. 59.

MAPK Inhibitor Removal–Driven pERK Rebound Synergizes with DNA Damage

- MAPKi removal induces either a cell-death or slow-cycling phenotype in MAPKi-resistant melanoma.
- Excessive ERK hyperactivation after MAPKi withdrawal induces DNA damage and parthanatos-mediated death.
- Targeting DNA repair pathways after MAPKi cessation may be efficacious against MAPKi-addicted melanoma.

Dual BRAF/MEK inhibitor therapy is initially efficacious in patients with melanoma, but frequently results in acquired resistance to MAPK inhibitors (MAPKi). Having previously shown that BRAFV600-mutant melanomas increasingly depend upon MAPKi as they adapt to dual BRAFi/MEKi therapy and that MAPKi withdrawal gives rise to increased pERK levels and decreased tumor cell viability, Hong, Moriceau, and colleagues sought to elucidate the mechanisms underlying melanoma responses to MAPKi withdrawal and determine the generality of these mechanisms to MEKi-resistant NRAS-mutant melanoma. MAPKi-resistant melanoma cell lines generally exhibited two distinct phenotypes after MAPKi withdrawal: a cell death–predominant drug addiction phenotype that was associated with low levels of MAPKi withdrawal-induced pERK rebound and dependent upon AIF activation and DNA damage induction; and a slow cycling–predominant drug addiction phenotype that was associated with low levels of MAPKi withdrawal–induced pERK rebound and dependent on p38-FRA1/JUNB. Treatment with DNA damage repair inhibitors during MAPKi withdrawal induced phenotypic switching from a slow-cycling to a CASP3-dependent apoptotic cell death phenotype in slow cycling–predominant MAPKi-resistant melanoma, whereas PARPi treatment augmented parthanatos-related cell death in cell death–predominant MAPKi-resistant melanoma. Vemurafenib paradoxically induced even greater pERK rebound, DNA damage, and tumor regression during MEKi withdrawal from MEKi-resistant melanoma with NRAS mutations or an atypical BRAF mutation. In NRAS-mutant patient-derived xenografts or syngeneic murine melanoma which have acquired MEKi resistance, dual PARPi/vemurafenib treatment after MEKi withdrawal resulted in the greatest and most sustained tumor regression. These findings describe a mechanism underlying MAPKi addiction in melanoma that can be therapeutically exploited.

See article, p. 74.
AMPK Regulates Nucleotide Synthesis to Sustain Tumor Growth

- AMPK phosphorylates PRPS1/2 in response to glucose deprivation or hypoxia.
- Phosphorylation converts PRPS1/2 hexamers to monomers and inhibits nucleotide synthesis and NAD production.
- Inhibition of PRPS1/2 conserves ATP and NADPH and promotes tumor growth under stress conditions.

Rapidly proliferating tumor cells upregulate production of nucleotides, amino acids, and lipids to meet increased bioenergetic demands and counteract stress caused by glucose-deprived or hypoxic environments. Hexameric phosphoribosyl pyrophosphate synthetase (PRPS) enzymes catalyze the production of phosphoribosyl pyrophosphate (PRPP), which is necessary for de novo synthesis of purine and pyrimidine nucleotides and coenzymes such as nicotinamide adenine dinucleotide (NAD).

Qian, Li, and colleagues observed that energy stress in glioblastoma cells induced by glucose deprivation or hypoxia led to a rapid decrease in PRPP levels, nucleotide synthesis, and NAD production that was caused by increased phosphorylation of PRPS1 and PRPS2 by AMP-activated protein kinase (AMPK), which converted PRPS1/2 hexamers to monomers and inhibited PRPS catalytic activity. AMPK-dependent phosphorylation of PRPS1/2 and subsequent suppression of nucleotide synthesis and NAD production conserved cellular ATP and NADPH (the reduced form of NAD phosphate that eliminates reactive oxygen species from cells) under energy stress, whereas expression of nonphosphorylatable PRPS1/2 mutants led to unrestricted nucleic acid synthesis and cellular ATP and NADPH consumption, which significantly increased levels of reactive oxygen species and induced apoptosis. Consistent with these findings, blockade of AMPK-mediated PRPS1/2 phosphorylation significantly increased the sensitivity of tumor cells to energy stress in vitro and reduced tumor growth in vivo, suggesting a critical role for AMPK in direct regulation of nucleotide synthesis and maintenance of cellular homeostasis during tumor growth.

See article, p. 94.

Distinct Noncoding and Coding Alterations Activate KLF5 in Tumors

- Focal amplification of a noncoding region near KLF5 recurs in human cancers and increases KLF5 expression.
- Hotspot coding mutations increase KLF5 protein stability or alter KLF5 DNA binding specificity.
- Increased KLF5 activity confers dependence on KLF5 for proliferation, implicating it as a potential target.

Kruppel-like transcription factor 5 (KLF5) is a zinc-finger DNA binding protein that has been shown to promote cell proliferation and tumorigenesis in multiple tissues. Genomic studies have identified recurrent missense mutations in KLF5 and copy-number alterations encompassing the KLF5 locus across several cancer types, but the phenotypic consequences are unclear. Zhang and colleagues evaluated the effect of KLF5 alterations on KLF5 gene expression, KLF5 protein stability, and KLF5 protein function. A focal amplification of an intergenic noncoding region adjacent to KLF5 included several large clusters of enhancers, known as superenhancers, that physically associated with the KLF5 promoter and specifically activated KLF5 gene expression. Within the coding region of KLF5, phospho-degron domain hotspot mutations were found to block binding of KLF5 by the E3 ubiquitin ligase FBXW7 and thus increase KLF5 protein stability, whereas DNA binding domain hotspot mutations altered KLF5 DNA binding specificity and led to a significant gain in novel KLF5 binding sites that created new superenhancers and increased expression of cancer-related genes. Of note, cancer cells harboring KLF5 superenhancer amplifications or with increased KLF5 expression were dependent on KLF5 for proliferation, suggesting that KLF5 may be a therapeutic target in tumors with increased KLF5 activity. Although the frequency of each type of KLF5 alteration alone is relatively modest, the combined frequency of genomic alterations functionally converging on increased KLF5 activity suggests that KLF5 may play a broader oncogenic role in human cancer than initially suspected.

See article, p. 108.