COMMENTARY

Targeting osteosarcoma

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Small-molecule targeted therapies have significantly improved the treatment landscape for many cancers. When a patient's tumor expresses the protein kinase BCR-ABL or the protein kinase BRAFV600E, or overexpresses the androgen receptor, we now know what to do: treat with the corresponding small molecule that targets the driver oncogene. However, in a so-called "orphan cancer" such as osteosarcoma (OS) with no known driver oncogenes, finding an effective targeted therapy is a tall order. In PNAS, Perry et al. (1) undertook this challenge by starting with state-of-the-art gene sequencing, following up with comparison to an OS mouse model, proceeding to uncover therapeutically tractable targets with a genome-wide reverse genetic screen, and finally validating the genetic hits with small-molecule drugs currently in clinical trials for various cancers. Perry et al.'s multifaceted approach identified the phosphatidylinositol 3-kinase/mammalian target of rapamycin (PI3K/mTOR) pathway as a potential new OS treatment target (1).

Genetic Complexity of OS

OS is the most common primary tumor of the bone and is an especially aggressive, predominantly pediatric cancer. The standard of care remains cytotoxic chemotherapies, yet the 60% 5-y survival rate has not improved since the 1980s (2). Although failing to identify driver oncogenes, previous next-generation sequencing efforts have instead revealed the vast genetic complexity of OS tumors (3). Most pediatric cancers typically have low somatic mutation rates around 0.1 mutations per megabase, whereas even those thought to have the highest somatic mutation rates are around 0.4–0.5 mutations per megabase. However, Perry et al. (1) found the median somatic mutation rate in OS to be an astounding 1.2 mutations per megabase, on the order of some adult cancers (4). Along with the low sample size that comes with a rare tumor, this high somatic mutation rate makes it quite hard to find anything of statistical significance upon sequencing. Knowing this, Perry et al. (1) combined sequencing data with several different analytical methods to increase statistical power. Pathway analysis based on the sequencing mutational profile validated the p53 and retinoblastoma (Rb) pathways, previously shown to be mutated in OS. Interestingly, despite no documented Li Fraumeni patients included in the analysis, the authors reported germ-line mutations in *TP53* at a frequency of 12%, four times the rate previously reported (5). If this result bears out in further studies, Perry et al. (1) note that perhaps patients with sporadic OS should be referred to genetic counseling to discuss testing for Li Fraumeni syndrome, which is not currently standard practice. A combination of geneset enrichment analysis with the Molecular Signatures Database identified new pathways of interest, many of which implicated PI3K/mTOR signaling (6). Subsequently, individual genomes were evaluated using the Precision Heuristics for Interpreting the Alteration Landscape algorithm, which ranks genetic alterations (including mutations, insertions and deletions, and copy number alterations) by clinical and biological significance, such that the most clinically relevant alterations are most rapidly identified (7). Thirty-four percent of the patients had mutations with immediate clinical implications using available approved drugs, with 24% of patients harboring alterations in the PI3K/mTOR pathway. Similar mutations were found by the team in a comparative oncology approach by sequencing tumor/normal pairs from a mouse model of OS (8). Finally, Perry et al. (1) performed a whole-genome scale shRNA screen in a primary cell line derived from murine OS for essential proliferation and survival genes. Again, their dataset turned up *Pik3ca* and *Mtor*.

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Based on the multiple analyses implicating the PI3K/mTOR pathway, Perry et al. (1) treated human and murine OS cell lines with two dual PI3K/mTOR inhibitors—GSK2126458 and BEZ235—as well as with the PI3Kα inhibitor PIK75. All three compounds potently inhibited proliferation of all OS-derived cell lines tested and induced apoptosis, with the exception of BEZ235, which was cytostatic. The authors noted that despite the universal sensitivity across all cell lines tested, at best only one had a PI3K/mTOR pathway mutation, demonstrating that mutation may not be necessary for sensitivity. This is further supported by the fact that Perry et al. did not find any PI3K/mTOR pathway mutations in their initial sequencing results. The subsequent analyses using a multimodal approach increased both the rigor of their study as well as the statistical power, and this is what sets Perry et al.'s paper apart from past studies implicating components of the PI3K/mTOR pathway in OS based on immunohistochemistry or phosphoprotein analysis (9–11). Furthermore, Perry et al.'s (1) model sets an excellent precedent for attacking other orphan cancers with no known driver oncogenes, and therefore no known effective targeted therapies.

In considering the signaling that underpins the reported results, perhaps it is not surprising that the PI3K/mTOR pathway emerged as the best therapeutic target. mTOR sits uniquely downstream of numerous signaling pathways required for a diverse set of important cellular survival and proliferation programs. Perhaps this convergence on mTOR functions as a signaling "choke point," creating an exquisite vulnerability and therefore opportunity for targeted therapies. This aspect is akin to what has previously been referred to as a molecular

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"funnel factor" in reference to phospho-eIF4E–binding protein 1 (p-4E-BP1), an mTOR substrate, regardless of upstream oncogenic alterations (12).

Discovering which cancers may benefit most from mTOR inhibition is an ongoing effort. There are currently 279 clinical trials based on mTOR inhibitors. The vast majority of these trials (225) use either rapamycin or one of several so-called rapalogs (synthetic analogs of rapamycin), despite the fact that their activity has been shown to be limited and inhibition incomplete, particularly for 4E-BP1 phosphorylation (13). Taking into account the model of mTOR and 4E-BP1 functioning as a choke point, we would not expect these drugs to prove very effective. To this end, there are now several ATP-competitive mTOR inhibitors in clinical trials (MLN0128, AZD2014, CC-223). Additionally, there is a handful of dual PI3K/mTOR inhibitors in clinical trials as well as one dual mTOR/DNA-PK inhibitor. Finally, we would be remiss if we didn't point out in a discussion of clinical therapeutics that with so many genetic perturbations, OS is a cancer that may respond well to immunotherapies, as have melanoma and other highly mutated cancers.

Moving Faster to the Clinic

It is still early days for the new generation of mTOR inhibitors in the clinic, and therefore the majority of them are still in single agent trials. As Perry et al. (1) point out in their report, it is extremely difficult to achieve statistical significance for a drug with such a small patient population available for enrollment. Perry et al.'s study resorted to a large number of samples from Brazil. Wouldn't it be great if we had another more rapid way to test drugs that might have benefit in OS? Such a model would ideally be a spontaneously occurring OS tumor that faithfully recapitulates the histology, genetic instability, and molecular pathology of human OS. Canine OS is the most common bone cancer in dogs and occurs about 10-times more frequently than in humans (14). However, are companion canines good predictors of whether new drugs will be effective in humans? It is a little-known fact that the BTK inhibitor Ibrutinib may not have been clinically tested for the treatment of B-cell malignancies without the promising data from a clinical trial in companion canines, who also get spontaneous B-cell lymphomas (15). There is a growing appreciation for the power of testing drugs in outbred populations of companion canines who experience the same environmental factors as human patients. Researchers at the University of Washington, Seattle recently made headlines for announcing a pilot study to investigate the ability of rapamycin to extend lifespans in dogs, as it has already been shown to do in flies, worms, and mice (16). Everybody benefits from investigating how to treat cherished family pets' cancers or increase their lifespans. With the increased incidence of OS in canines, these trials would be easier to enroll, and ultimately identify promising treatments for pediatric patients faster. Most importantly, the key hallmark of OS identified in the Perry et al. (1) study was the extreme genetic heterogeneity of this rare cancer, a feature known to be shared with the canine version of OS (17).

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