Screening for functional kinases in metastatic prostate cancer: a glimmer of hope for kinase inhibition

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Understanding the molecular biology of castration-resistant prostate cancer (CRPC) has led to a dramatic paradigm shift in the treatment of patients with metastatic disease where the androgen receptor (AR) is a central therapeutic target in the disease. Despite these major advances, disease biology and the well-recognized heterogeneity of prostate cancer (PCa) are some of the inherent disease-specific issues that continue to affect clinical trial design, drug development and ultimately patient outcomes. Perhaps missing in PCa therapeutics is the revolution other cancers have experienced with the design and development of novel agents capable of targeting wild-type and mutated kinases implicated as drivers of disease development, resistance and progression. The landmark discovery of BCR-ABL in the 1980s and the subsequent development of imatinib in chronic myelogenous leukemia (CML) transformed the therapy of a previously fatal disease (1,2). Kinase inhibition has since revolutionized drug discovery and development in a variety of cancer subtypes including BRAF in metastatic melanoma, EGFR and ALK in non-small cell lung cancer, and BTK in lymphoma (3-5). In all these examples, the dramatic clinical responses with these agents have reshaped the face of the diseases and their treatment paradigms.

Recent efforts characterizing the genome of CRPC have demonstrated the heterogeneity and molecular complexity of the disease (6). Although a significant number of genomic aberrations have been reported, their precise roles in the pathogenesis and progression to a CRPC state are not entirely clear. These exciting data are somewhat obscured by the lack of clinical benefit observed when novel kinase inhibitors were utilized in clinical trials of men

with CRPC (7-9). Perhaps most notably were the negative results of a large international phase III trial evaluating the combination of docetaxel and dasatinib, a Src inhibitor, to standard docetaxel in men with mCRPC (10).

There are a variety of possible explanations for the failures observed in these biologically driven clinical trials. It is possible that the functionality of the targets in question remains undiscovered in PCa or that the functional contribution is relatively minor or clinically insignificant. Moreover, challenges in obtaining tissue biopsies before and after treatment with novel therapies have limited investigators' abilities to further define the molecular and clinical impact of kinase inhibition Additionally, the relationship and interaction between cytotoxic therapy and kinase inhibitors is unknown and it is conceivable that the use of docetaxel in this setting could abrogate the benefit of kinase inhibition.

In a recently reported study, Faltermeier and colleagues set out to screen for and identify those kinases that functionally could drive visceral and bone PCa metastasis (11). The authors systematically reviewed previously described phosphoproteomic and transcriptome datasets as well as the general literature to identify those kinases likely to promote progression of PCa. Of more than 500 kinases encoded by the human kinome, the authors identified 125 kinases for further analysis.

They subsequently performed a gain-of-function screen in which kinases were overexpressed in murine Cap8 cells and injected into mice. The Cap8 cells also were also tagged with a luciferase-encoding vector to monitor *in vivo* metastasis with bioluminescence imaging (BLI).

BLI-identified lung metastases were subsequently excised and analyzed by western blot to determine specific kinase enrichment. Of the 125 kinases investigated, 20 were overexpressed in metastatic tissue and thus deemed by the authors as possible "enhancers of metastasis". To determine which of these 20 kinases are "drivers of metastasis", the authors used non-malignant human prostate cells from the RWPE-1 cell line. Each of the 20 kinases was individually introduced into luciferase-expressing RWPE-1 cells which were then injected into mice. The authors noted that mice injected with RWPE-1 cells expressing ARAF, BRAF, CRAF, MERTK, NTRK2 kinases developed hind-leg weakness between 1–6 months after injection and BLI signals highlighted disease in the hind legs. Moreover, PET-CT imaging demonstrating ^{18}F-FDG uptake in the lungs, bones, and lymph nodes suggest a critical role for these kinases in the metastatic process (11).

Radiographic evidence of disease was subsequently confirmed by histological evaluation of tissue from these metastatic sites. To confirm that the bone metastases were in fact a product of the human RWPE-1 cell line, immunohistochemical (IHC) detection of the individual kinases as well as HLA, PSA, and epithelial cell marker E-cadherin were confirmed in bone metastases from these mice. Finally, the authors analyzed samples of human metastatic PCa tissue and non-malignant human prostate tissue and found overexpression of ARAF, BRAF, CRAF, NERTK, and NTRK2 kinases in the malignant samples. Taken together, these data suggest that these five kinases are critical in the development of visceral and bone metastases in PCa (11).

The authors should be commended for the elegant design of their study and their thoughtful and systematic approach through which they identified these five kinases. These experiments strongly suggest that overexpression of specific wild-type kinases promote progression to metastatic PCa.

Despite the sophistication of their study, however, many critical questions remain unanswered. The focus of this study is wild-type kinases with little mention of mutated kinases that, although very rare, do exist and may have a role in the development of metastatic PCa (6,12,13). It is also unclear whether the overexpression of the wild-type kinases is itself responsible for disease progression or whether there is a background of kinase mutations driving these changes. Similarly, the translation of this research to the clinic remains uncertain. To date, clinical trials evaluating tyrosine kinase inhibitors capable of inhibiting three of the five kinases implicated in this study (ARAF, BRAF, and

CRAF) have failed to show clinical benefit in men with mCRPC (7,9). Most recently, the phase III trial results of cabozantinib, a dual MET and VEGFR TKI also failed to demonstrate a survival benefit in men with mCRPC despite the well-established role of VEGF and MET in metastatic PCa (14-17).

Furthermore, this study identifies those kinases that are critical for metastatic progression. In considering potential therapeutic implications of this research, there are a number of challenges particularly regarding choice of patients. Should clinical trials be done on patients with local disease to prevent metastases? Should clinical trials instead be focused on patients with biochemical recurrence after definitive local therapy? Might there be a benefit for kinase inhibitor use in patients with radiographic evidence of metastatic PCa to prevent further metastases or skeletal-related events? These challenges were demonstrated in the phase III trial comparing docetaxel and atrasentan versus docetaxel alone in patients with mCRPC who had bone metastases (18). Given that atrasentan is an endothelin receptor antagonist with preclinical data demonstrating osteoblastic metastatic inhibition in PCa (19), it was hypothesized that its use in this population could improve outcomes. Unfortunately, the study yielded negative results thus highlighting many of the struggles between biologic and clinical endpoints.

There are a number of possible explanations for the discrepancy between the link between kinases and PCa progression in laboratory models and a lack of significant clinical benefit in clinical trials. Patient selection is perhaps the most important factor to identify those likely to respond to kinase inhibition. Although kinase inhibitor clinical trials have included all patients who met global inclusion criteria, the benefit of kinase inhibition in PCa may be limited only to those patients who have kinase overexpression or overactivity. Future clinical trials may need to screen for kinase expression or activity as an inclusion criterion for entry to such trials.

Another possible explanation relates to the generally complex network of kinases. Kinases exist as part of intricate pathways in which the inhibition or overexpression of a single kinase induces a cascade of downstream effects. The clinical trials of kinase inhibitors in PCa have thus far focused on kinase inhibition with single agent kinase inhibitors. However, it is reasonable to consider that perhaps success in PCa kinase inhibition will only be discovered using combination therapy targeting multiple kinases along a particular pathway. This could mimic

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the impressive results seen in combination therapy for metastatic melanoma using the combination of a BRAF inhibitor (dabrafenib) with an inhibitor of its downstream MEK kinase (trametinib) (20,21).

In summary, although patients with mCRPC have more available therapies today than just a decade ago, there remains significant room for improvement in the treatment strategies for this cohort of patients. Kinases that are overexpressed in PCa are logical hypothetical therapeutic targets especially given their clear implication in advanced disease as demonstrated by Faltermeier and colleagues (11). Although the clinical implications of this study's findings remain uncertain and have yet to bear fruit in other clinical trials, they provide an optimistic outlook for the potential development and incorporation of kinase inhibitors for the treatment of advanced PCa.

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