Identifying Actionable Targets through Integrative Analyses of GEM Model and Human Prostate Cancer Genomic Profiling

Jackie Wanjala1, Barry S. Taylor2, Caren Chapinski1, Haley Hieronymus1, John Wongvipat1, Yu Chen1,5, Gouri J. Nanjangud1, Nikoalns Schultz2, Yingqiu Xie5, Shenji Liu5, Wenfu Lu5, Qing Yang5, Chris Sander2, Zhenbang Chen5, Charles L. Sawyers1,6, and Brett S. Carver1,7

Abstract

Copy-number alterations (CNA) are among the most common molecular events in human prostate cancer genomes and are associated with worse prognosis. Identification of the oncogenic drivers within these CNAs is challenging due to the broad nature of these genomic gains or losses which can include large numbers of genes within a given region. Here, we profiled the genomes of four genetically engineered mouse prostate cancer models that reflect oncogenic events common in human prostate tumors, with the goal of integrating these data with human prostate cancer datasets to identify shared molecular events. Met was amplified in 67% of prostate tumors from Pten p53 prostate conditional null mice and in approximately 30% of metastatic human prostate cancer specimens, often in association with loss of PTEN and TP53. In murine tumors with Met amplification, Met copy-number gain and expression was present in some cells but not others, revealing intratumoral heterogeneity. Forced MET overexpression in non-MET-amplified prostate tumor cells activated PI3K and MAPK signaling and promoted cell proliferation and tumor growth, whereas MET kinase inhibition selectively impaired the growth of tumors with Met amplification. However, the impact of MET inhibitor therapy was compromised by the persistent growth of non-Met-amplified cells within Met-amplified tumors. These findings establish the importance of MET in prostate cancer progression but reveal potential limitations in the clinical use of MET inhibitors in late-stage prostate cancer.

Introduction

The development of high-throughput genomic platforms has allowed comprehensive profiling of human malignancies with the goal of identifying oncogenic driver events that may impact patient management. Studies profiling primary and metastatic prostate cancer have identified a number of established and novel oncogenic events in prostate cancer, including loss of the tumor suppressors PTEN and TP53, genomic rearrangements of ERG, amplification of MYC, focal loss of chromosome 3p, and mutations in SPOP (1–3).

Genomic studies efficiently identify oncogenes or tumor suppressors when specific genes are frequently mutated or have focal gain or loss. However, the utility of this approach is limited when broad regions of genomic gain or loss are present because of the large number of genes impacted. Furthermore, these broad genomic gains and losses could simply be a consequence of genomic instability rather than playing a causal role in the cancer. Interestingly, tumors that develop in genetically engineered mouse (GEM) models often do not have the same complexity of copy-number alterations (CNA) as their human counterparts, unless these mice are crossed into strains with genetically unstable backgrounds (4). The relative simplicity of murine tumor genomes provides a convenient opportunity to conduct integrative mouse-human tumor genomic analysis to identify critical human tumor drivers (5, 6).

Several GEM models of prostate cancer have been developed which faithfully recapitulate the oncogenic driving genomic alterations and histopathology of prostate cancer. Although these model systems to date have not faithfully recapitulated the metastatic disease process, several of these GEM models indeed model genetic events enriched in both primary and metastatic prostate cancer. We elected to use 4 GEM models most representative of the common genomic alterations present in prostate cancer. PB-MYC mice model amplification and overexpression of MYC observed in human prostate cancer (7). These mice develop high-grade prostatic intraepithelial neoplasia (HGPIN) by 2 months of age, which progresses to established invasive adenocarcinoma by 12 months of age. Pten prostate conditional null mice (Ptenlox/lox PB-Cre) develop HGPIN by 2 months of age, which progresses to intraductal carcinoma by 6 months of age (8).
GEM modeling of prostate conditional loss of both Pten and Tp53 (Ptenlox/lox P53lox/lox PB-Cre), two genomic events enriched in metastatic prostate cancer, results in a rapidly progressive invasive carcinoma at 2 months of age and a lethal phenotype secondary to local invasion of adjacent organs by 6 months of age (9). Finally, mouse models displaying prostate conditional loss of Pten and overexpression of ERG, modeling the ERG genomic rearrangements present in 50% of prostate cancers (Rosa26lox-stop-lox ERG Ptenlox/lox PB-Cre) demonstrate HGPIN by 2 months of age with progression to invasive adenocarcinoma by 6 months of age (10).

Here, we applied this integrative mouse-human tumor genomics strategy to prostate cancer. Using these four GEM models that reflect common driver alterations found in human tumors (7–10), we found that the gene encoding the Met receptor tyrosine kinase was frequently amplified in murine prostate tumors initiated by loss of the tumor suppressors Pten and p53. Analysis of publicly available genomic data sets of human prostate cancer revealed MET amplification in approximately 30% of metastatic cases but rarely in primary tumors. Importantly, Met copy-number gain and expression in Met-amplified tumors is heterogeneous. Thus, although MET inhibition impairs the growth of MET-amplified tumors, intratumoral heterogeneity compromises long-term therapeutic efficacy. These findings have implications for ongoing clinical trials of MET inhibitors in advanced prostate cancer.

Materials and Methods

Mouse models of prostate cancer

The GEM models of human prostate cancer (PB-MYC, Ptenlox/lox PB-Cre, Rosa26lox-stop-lox ERG Ptenlox/lox PB-Cre) used in our experiments were maintained in our animal housing facility in accordance with our IACUC protocol, and experiments involving Ptenlox/lox P53lox/lox PB-Cre were conducted in collaboration with Zhenbang Chen, PhD, at Meharry Medical College in accordance with the IACUC protocol. All mice were genotyped according to established protocols (7–10). Tumor tissues were harvested for molecular profiling at 18 months of age for PB-MYC mice, 12 months of age for Ptenlox/lox mice, 6 months of age for ERG Ptenlox/lox mice, and 6 months of age for Ptenlox/lox P53lox/lox mice. The tissue was macrodissected to enrich for epithelial cancer cells. Genomic DNA and RNA were isolated from 30 mg of prostate cancer tissue using the DNeasy Blood and Tissue Kit (Qiagen) and TRIzol (Invitrogen) extraction followed by purification with the RNasy Mini Kit (Qiagen). Protein was harvested from prostate cancer specimens by digestion with RIPA buffer.

Cell lines

The cell lines used in our experiments included the MYC CaP line derived from a PB-MYC mouse by Sawyers’ lab, the Cap8 line derived from a Ptenlox/lox from the Wu lab, the MPC3 line derived from a Ptenlox/lox P53lox/lox from the Chen lab, and the LAPC4 parental line generated by the Sawyers’ lab. These lines have been maintained by our lab. LAPC4 cells have been authenticated using DNA HapMap genotyping. The MYC CaP, Cap8, and MPC3 lines have been authenticated by DNA genotyping of the transgene and recombination oflox sites.

Molecular profiling

Array comparative genomic hybridization. DNA was isolated, quantified using the Thermo Scientific NanoDrop 1000 Spectrophotometer, and submitted to our Genomics Core Laboratory for array comparative genomic hybridization (CGH) analysis for 16 PB-MYC mice, 11 Ptenlox/lox mice, 7 ERG Ptenlox/lox mice, and 18 Ptenlox/lox P53lox/lox mice. In addition, DNA prepared from the GEM model–derived cell lines, MYC CaP (PB-MYC), Cap8 (Ptenlox/lox), and MPC3 (Ptenlox/lox P53lox/lox), was submitted for CGH analysis. Reference DNA was prepared from the prostate of genotype/strain matched wild-type littermate mice. All DNA met the requirements of an A260/280 ratio of 1.6 to 1.8 and a concentration greater than 150 μg/mL. Three micrograms of tumor and reference DNA was digested and labeled by random priming using the Bioprime Kit (Invitrogen). Labeled DNA was hybridized to the mouse Agilent 244K CGH array, and the slides were scanned and images quantified using Feature Extraction 9.1 (Agilent). Raw data from the Agilent mouse 244K CGH array were normalized as previously described, and probe level data were segmented with Circular Binary Segmentation and analyzed with RAE.

Transcriptome profiling. Total RNAs were isolated and quantified using the Thermo Scientific NanoDrop 1000 Spectrophotometer. RNA samples with an A260/280 ratio of >1.8 and concentration greater than >20 ng/μL were submitted to our Genomics Core Laboratory from prostate tumors profiled by CGH of 5 PB-MYC mice, 5 Ptenlox/lox mice, and 14 Ptenlox/lox P53lox/lox mice. Quality of the RNA was assessed by the RNA 6000 picoAssay, and a RIN number >7 was considered adequate for labeling. A total of 300 ng of RNA was labeled and hybridized on the Illumina MouseRef-8 v2 bead arrays. Raw data were log, transformed and normalized. Gene Set Enrichment Analysis (GSEA) was performed to determine gene sets disproportionally over- or underexpressed in groups stratified by specific CNAs. Hierarchical clustering was carried out on genes meeting the criteria of greater than 3-fold change across more than two samples using Pearson correlation with pairwise complete linkage.

FIISH analyses

FIISH analysis was performed on formalin-fixed paraffin-embedded (FFPE) sections using a two-color probe mix. The probe mix consisted of BAC clones containing the full-length Met gene (locus 6q42; clones RP23-173P9 and RP23-444N4; labeled with Red dUTP) and a proximal centromeric locus which serves as the control (locus 6q41; clones RP23-258F1 and RP23-355D10; labeled with Green dUTP). Probe labeling, hybridization, posthybridization washing, and fluorescence detection were performed according to standard procedures. Slides were scanned using a Zeiss Axioplan 2i epifluorescence microscope equipped with a megapixel CCD camera (CV-M4 CL, JAI) controlled by Isis 5.2 imaging software (Metasystems Group Inc.). Entire sections were scanned under 6× objective to assess heterogeneity for Met copy number or amplification and in each representative region, a minimum of 50 to 100 nuclei scored. Amplification was defined as Met:6q41 (Control) ratio of >2.2 or >10 copies of Met independent of control locus. Cells with 3 to 6 copies and 7 to 10 copies of Met and control locus were considered to be polysomic and high-polysomic, respectively.

Human prostate cancer molecular profiling

The human prostate cancer data sets used herein have been previously published (3). The complete genomes dataset and analytic methods are reported separately and are available at
The LAPC4 cell lines were infected and selected with RFP control.

In vitro experiments
In vitro experiments were conducted using the LAPC4 cell line. The LAPC4 cell lines were infected and selected with RFP control and MET-expressing virus and selected with puromycin. Proliferation assays were conducted by plating 1 × 10^5 cells per well of a 12-well cell culture plate and counted using Cell Titer Glo on days 1, 3, and 5. HGF stimulation of LAPC4 cells was conducted over a time course with 50 ng of HGF. Crizotinib (Pfizer), an MET inhibitor, was obtained through a materials and transfer agreement with Pfizer and administered to LAPC4 cells at defined concentrations for 24 hours for protein evaluation and as part of a cell proliferation assay. Cell lysates for Western blot analysis were prepared using standard RIPA buffer. All experiments were conducted in triplicate and SDs were reported.

Preclinical in vivo studies
Xenograft model. For xenograft experiments, 1 × 10^6 LAPC4-RFP and LAPC4-MET cells were injected into the bilateral flanks of SCID mice (10 mice per group) and tumor volumes were measured weekly over 28 days. At the end of study, mice were euthanized and tumor tissue was procured for FFPE. Representative slides were evaluated by hematoxylin and eosin (H&E) staining and used for immunohistochemical analyses.

GEM prostate cancer transplant model. Prostate tumors from Pten p53 null mice were harvested at 6 months of age, dissected into 2 × 2 × 2 mm cubes, and transplanted into the unilateral flank of athymic mice. Growth of these tumors was demonstrated by measuring tumor volume over 16 days. Animals were euthanized and specimens were procured for FFPE. Transplanted mice were randomized to receive vehicle control or crizotinib (Pfizer; 50 mg/kg/day), and tumor volumes were measured weekly over 21 days. At the end of study, mice were euthanized and tumors were procured by FFPE for H&E and immunohistochemical analyses. Similar studies were conducted using our Rosa-26-ERG Ptenlox/lox, which by aCGH do not demonstrate copy-number gain of Met.

Immunohistochemical and Western blotting antibodies
The antibodies used for Western blot analysis and immunohistochemistry were pAKT Ser473 (Cell Signaling Technology; 1:1,000 dilution), pAKT Thr308 (Cell Signaling Technology; 1:1,000 dilution), AKT (Cell Signaling Technology; 1:1,000 dilution), pS6 Ser240/244 (Cell Signaling Technology; 1:1,000 dilution), pERK Thr202/Tyr204 (Cell Signaling Technology; 1:1,000 dilution), ERK (Cell Signaling Technology; 1:1,000 dilution), pMET Tyr1234/1235 (Cell Signaling Technology; 1:1,000 dilution), pMET Tyr1003/1004 (Cell Signaling Technology; 1:1,000 dilution), MET (Cell Signaling Technology; 1:1,000 dilution), Pten (Cell Signaling Technology; 1:1,000 dilution), PIK3CA/B (Cell Signaling Technology; 1:1,000 dilution), and P53 (Santa Cruz Biotechnology; 1:200 dilution). All immunohistochemical analyses were conducted by the MSKCC Molecular Cytology core.

Accession number
NIH NCBI Gene Expression Omnibus (GEO) Agilent aCGH GEO accession number is GSE61380. Illumina microarray expression GEO accession number is GSE61379.

Results
Recurrence amplification of Met in Pten/p53 null murine prostate tumors
Prostate cancer specimens harvested from PB-MYC, Ptenlox/lox, Ras26-ERG Ptenlox/lox, and Ptenlox/lox p53lox/lox mice were profiled by array CGH (Agilent) to identify secondary acquired genomic alterations (Fig. 1A; Supplementary Table S1). CNAs were analyzed using the RAE method and revealed recurrent amplifications and deletions across a number of chromosomal regions (Fig. 1B; Supplementary Table S2). Unsupervised clustering of CNAs demonstrated that the majority of genomic changes were in specimens derived from Ptenlox/lox p53lox/lox mice (Supplementary Fig. S1). Regions of focal amplifications spanning known or putative oncogenes included 4q(C5) (Jun), 6qA2 (Met), 7qF3 (Fgf2), and 9qA1 (Yap1, Mmp3, Mmp7; Fig. 1B and C). Copy-number gains spanning Met and Jun were focal in some tumors (Fig. 1C). Overall, 12 of 18 (67%) prostate cancer specimens from Pten p53 null mice demonstrated gain of Met. In addition, broad regions of amplification were observed for mouse chromosomes 5 and 15, which are syntenic for human chromosomes 7 and 8q and are broadly amplified in human prostate cancer (Supplementary Figs. S1 and S2). Recent work from Ding and colleagues (4) reported that re-expression of mTert in the prostate of Pten p53 null mice resulted in the accumulation of secondary genomic alterations. In a direct comparison of our copy-number data with their published data, the CNAs were significantly concordant with the exception of 3 chromosomal regions (Supplementary Fig. S2). Therefore, mTert overexpression is not required for the development of secondary genomic alterations in this model, likely due to the impact of p53 loss.

Enrichment of MET/HGF copy-number gain in castration-resistant human prostate cancer
Several genomic profiling studies of human prostate cancer failed to recognize MET amplification as a frequent event (2, 3). On the basis of the association of Met amplification with Pten/p53 loss in murine prostate cancer, we reanalyzed the copy-number data from these studies, taking a focused look in metastatic prostate cancer cases when PTEN and TP53 are frequently deleted. Remarkably, copy-number gain involving MET was present in 32% of metastatic prostate cancer (3). MET amplification was significantly associated with concomitant copy-number gain of the MET ligand HGF (present in 35% of all cases and in 83% of MET-amplified cases; P value < 0.001; Fig. 2A). Thirteen of 15 (87%) cases with MET/HGF gain also had alterations of the PI3K pathway (PTEN loss, INPP4B loss, PHLP1/2 loss, AKT1/2/3 copy-number gain, PIK3CA/B copy-number gain/mutation; Fig. 2B). These findings were validated in an independent dataset of castration-resistant metastatic prostate cancers (Fig. 2C; ref. 2). MET gain or mutation was present in 25% of cases and was significantly associated with gain of HGF (P < 0.001). Importantly, cases with MET amplification were enriched for gene expression signatures of MET pathway activation (FDR < 0.1) and HGF stimulation (FDR < 0.1), signifying that tumors with MET amplification are associated with a biologic signature of MET activation.
(Fig. 2D and E). In contrast to metastatic disease, copy-number gain of MET or HGF was rarely observed (4%) in primary prostate cancers.

Because copy-number gain of HGF was associated with MET amplification in human metastatic prostate cancer, we asked if this same association was present in mouse tumors. Three of 11 mouse tumors with Met amplification had regional gain of Hgf and others had increased expression of Hgf in the absence of Hgf gene amplification (Supplementary Fig. S3A). Interestingly, 2 tumors had copy-number gain of Hgf without Met amplification.

We also found that two other focally amplified genes in mouse tumors, Jun and Yap1, were also amplified in 3% to 5% and 10%
to 11% of human metastatic prostate cancers, respectively (Fig. 2A and C). Thus, analysis of murine prostate cancer genomes resulted in the identification of molecular events in human prostate cancer that are easily overlooked by "human only" genome studies.

**Met amplification is heterogeneous within individual tumors**

To evaluate the impact of Met amplification on Met expression, protein and RNA were harvested from our murine prostate tumor specimens previously analyzed by array CGH. Expression analysis (Illumina) was performed in a subset of prostate cancer specimens analyzed for CNAs. As expected, unsupervised clustering of genes differentially regulated across the GEM tumor specimens was primarily driven by genotype (Supplementary Fig. S3B). In accordance with the loss of PTEN and TP53 being enriched in human metastatic prostate cancers, modeling loss of Pten and p53 in GEM models of prostate cancer revealed enrichment of gene signatures associated with metastasis, although these murine tumors do not display a metastatic phenotype (Supplementary Table S3). Although this is likely secondary to differences in the biology of mouse and humans, and potentially the acquirement of secondary drivers of metastasis, our study reveals that there is molecular similarity when modeling metastatic alterations in primary murine prostate cancers, and thus may provide a pre-clinical model system to study therapies targeting metastatic prostate cancer.

Tumors with high-level Met copy-number gain had increased Met gene expression when compared with mice without high-level copy-number gain (Fig. 3A, P = 0.0002) and were enriched by GSEA for a Met signaling gene signature (Fig. 3B, FDR < 0.15). Western blot analysis of the prostate cancer specimens from Pten-deficient mice (Fig. 2E) revealed that HGF-induced Met tyrosine phosphorylation was increased in Pten-deficient tumors compared with tumors from wild-type mice. These results suggest that Met amplification may contribute to the enhanced HGF-dependent growth and survival of Pten-deficient prostate cancer cells.
Figure 3.
Genomic gain of Met is a heterogeneous-selected secondary event. A, Met mRNA expression was annotated by Met copy-number status for individual mice. Met mRNA upregulation was observed in individual cases with Met amplification. B, Pten p53 null prostate tumor specimens stratified by Met copy-number gain demonstrated enrichment for gene signatures of MET pathway activation. C, Western blot analysis of prostate cancer specimens from Pten p53 null mice demonstrate upregulation of Met in individual cases associated with high-level copy-number gains. D, heterogeneity of Met expression by immunohistochemistry. E, Western blot analysis of Met-amplified tumor specimens from Pten p53 null mice demonstrating heterogeneity of Met expression between and within individual tumors. F, representative FISH analysis for Met copy number in tumors from Pten p53 null mice demonstrating copy-number heterogeneity. Red color, Met; green color, control; blue color, centromeres, heterochromatin of mouse chromosomes.
p53 null mice revealed upregulation of Met in some but not all cases with copy-number gains (Fig. 3C). We hypothesized that the lack of increased MET protein expression in some tumors with Met copy-number gain might be explained by tumor heterogeneity. To evaluate this, we initially performed immunohistochemistry for Met in Met-amplified cases and observed heterogeneous Met expression both within and between different tissue sections of the same tumor (Fig. 3D). We then analyzed different regions of tumor specimens from Pten p53 prostate condition null mice harboring Met amplification by Western blot analysis and observed inter- and intratumor variability in Met protein expression (Fig. 3E). Downstream PI3K signaling, as measured by phosphoAKT levels, correlated with levels of Met phosphorylation. To directly evaluate intratumor heterogeneity with regard to Met CNAs, we conducted FISH analysis. In concordance with Met expression findings, we also observed intratumor heterogeneity in Met copy-number gain (Fig. 3F). Of 8 tumors analyzed, 2 were primarily diploid for Met, 3 showed foci of low-level copy-number gain, and 3 showed foci of high-level copy-number gain (amplification).

Overexpression of MET activates PI3K and enhances proliferation

MET plays a critical role in regulating cellular pathways influencing cell proliferation, cell migration, invasion, and morphogenesis (11–17). To determine the effect of MET overexpression in prostate tumors, we engineered LAPC4 cells, which lack MET amplification, to express elevated levels of MET. MET overexpression resulted in activation of the PI3K and MAPK downstream signaling pathways as measured by pAKT and pERK (Fig. 4A). Concordant with these findings, Met activation was associated with increased pAkt in prostates from Pten p53 null mice (Fig. 4B). Overexpression of MET in LAPC4 cells also promoted cell proliferation in vitro (P value < 0.001; Fig. 4C). To explore the impact of MET overexpression in vivo, LAPC4 xenografts were established in SCID mice. Tumors derived from MET-overexpressing cells grew faster than controls (P value < 0.001; Fig. 4D), consistent with increased staining for Ki67 by IHC (Fig. 4E).

HGF, the only known ligand of MET, has become increasingly recognized as a mediator of disease progression even in the absence of MET or HGF amplification. For example, autocrine or paracrine stimulation of MET by HGF confers resistance to tyrosine kinase inhibitors (18–20). Given that in human prostate cancer we observed a small subset of cases with copy-number gain of HGF without gain of MET, we asked if excess HGF was sufficient to promote cell proliferation. Stimulation with HGF promoted downstream signaling and proliferation in the setting of endogenous low levels of Met expression (P value = 0.03; Fig. 4F and G). Furthermore, stimulation of MET low cells with HGF conferred a growth advantage nearly comparable with that seen with MET overexpression (P value = 0.08).
MET kinase inhibitors impair the growth of Met-amplified tumors

Several MET inhibitors have entered clinical trials across a variety of malignancies (21, 22). We elected to use the MET/ALK inhibitor crizotinib because this drug is relatively selective compared with other clinical MET inhibitors such as cabozantinib (21, 23). To evaluate the therapeutic efficacy of MET inhibition, we treated LAPC4-MET cells with increasing concentrations of crizotinib and observed that cell proliferation was significantly reduced at a dose of 1 μmol/L (P value < 0.01; Fig. 5A). Furthermore, a dose of 1 μmol/L of crizotinib resulted in potent inhibition of MET and downstream kinase signaling (Fig. 5B and C).

We then turned to our Pten p53 null prostate cancer model where endogenous Met is frequently amplified. Given the heterogeneous expression of MET in these Met-amplified tumors, we predicted that MET inhibition would impact tumor growth but that low MET-expressing cells may display relative resistance. To generate sufficient animals to conduct this preclinical trial, we established a protocol for tissue grafting prostate cancer specimens directly from the GEM model (Supplementary Fig. S4A).

These tumors were harvested and dissected into 2 × 2 × 2 mm³ sections and grafted into the flank of athymic mice (Fig. 5D). Over a 14-day period, the tumors engrafted and grew rapidly. These tumors were further passaged into athymic mice, then treated with the MET inhibitor crizotinib (50 mg/kg/day) or vehicle control. Tumors from Pten p53 null mice, dissected, and transplanted into athymic mice to establish tumors. Mice with tumors were treated with vehicle (n = 8) or crizotinib (n = 8; 50 mg/kg/day; Pfizer) for a total of 21 days, and tumor volumes were measured on a weekly basis. Mice treated with crizotinib had a significant reduction in tumor growth. E, at the end of study, prostate tumors from mice treated with crizotinib had lower levels of cMet, pAkt, and ki67 staining by immunohistochemistry.
the greatest response (Fig. 6A–C). Of note, withdrawal of crizotinib resulted in accelerated tumor progression and reactivation of Met signaling, indicating that the Met-amplified cells may grow faster than the non–Met-amplified tumor cells and importantly that crizotinib does not deplete the Met-amplified cells of Met pathway dependency (Fig. 6D and E).

Discussion

Through the genomic profiling of prostate cancer GEM models, we identified several recurrent molecular events that develop during the evolution of murine prostate cancer. Integrating these murine data with genomic data from human prostate cancer led us to identify MET amplification as a molecular alteration with a high probability of playing a functional role in prostate cancer progression. Importantly, MET amplification did not emerge as a prostate cancer driver lesion using traditional human tumor genome analysis pipelines. However, reevaluation of the human tumor data based on our discovery of Met amplification in murine tumors revealed that approximately 30% of metastatic tumors have MET copy-number gain (2, 3). Further evidence for a biologic role of MET in prostate cancer is the fact that tumors with MET gain are statistically enriched for copy-number gains of the MET ligand HGF. Our biologic studies of forced MET overexpression and of pharmacologic MET inhibition further implicate MET as a driver of prostate cancer progression and a therapeutic target. However, the significant heterogeneity of MET expression within individual Met-amplified tumors may limit the effectiveness of MET inhibitors in advanced prostate cancer.

In this context, it is worth noting recent clinical results with the dual MET/VEGFR2 inhibitor cabozantinib (XL184) showing dramatic resolution of bone scan abnormalities in 86% of patients but soft tissue responses and serum PSA declines in only 25% to 30% of patients (22, 24, 25). This discrepancy suggests the bone scan effect of cabozantib may occur through inhibition of a target in the bone microenvironment, whereas the antitumor effect may be a consequence of target inhibition in tumor cells. Our data offer a potential explanation for these discrepant clinical response
rates, specifically that MET amplification could be associated with tumor response but not bone scan response. One prediction from our data is that MET inhibition would inhibit tumor growth only in the 20% to 30% of patients with MET amplification, a number consistent with the approximately 25% of patients with clinical soft tissue responses and serum PSA declines. Our data also suggest that these responses may be short lived due to the intratumoral heterogeneity of Met amplification we observed in the mouse tumors.

In summary, our work demonstrates the potential of integrated human and GEM model genomic profiling of prostate cancer to provide insight into new biologic drivers acquired during tumor progression, as illustrated by our discovery of Met amplification. Additional genomic alterations uncovered by this analysis, such as Jun and Yap1, also merit further evaluation.

Disclosure of Potential Conflicts of Interest
C.L. Sawyers is a consultant/advisory board member for Novartis, Agios, Blueprint, Beigene, Nextech, Tracon, and Housey Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions
Conception and design: J. Wanjala, Y. Chen, C.L. Sawyers, B.S. Carver
Development of methodology: J. Wanjala, B.S. Taylor, Y. Chen, S. Liu, C.L. Sawyers, B.S. Carver
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Wanjala, J. Wongvipat, Y. Xie, S. Liu, W. Lu, Q. Yang, Z. Chen, B.S. Carver
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Wanjala, B.S. Taylor, H. Hieronymus, Y. Chen, N. Schultz, Z. Chen, C.L. Sawyers, B.S. Carver
Writing, review, and/or revision of the manuscript: J. Wanjala, B.S. Taylor, C.L. Sawyers, B.S. Carver
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Wanjala, B.S. Carver
Study supervision: C. Sander, C.L. Sawyers, B.S. Carver
Other (obtained data): C. Chapinaki
Other (performed FISH experiment and analysis): G.I. Nanjangud
Other (performed the mouse experiments): Y. Xie
Other (involved in performing of the mouse experiments): W. Lu

Acknowledgments
The authors thank the Genetically Engineered Mouse Facility for maintaining mouse colonies. They also thank P.P. Pandolfo and Z. Chen for providing the Ptenfllox/lox p53fllox PB-Cre GEM model and all members of the Sawyers lab for providing informative discussion.

Grant Support
B.S. Carver is funded in part through RP-2 NIH Prostate SPORE P50-CA92629, NCI R01-CA182503, and has a Prostate Cancer Foundation Young Investigator Award. C.L. Sawyers is a Howard Hughes Medical Institute Investigator. Z. Chen is supported by Meharry Medical College MD004038.

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Received July 15, 2014; revised September 22, 2014; accepted October 21, 2014; published OnlineFirst November 7, 2014.

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Published OnlineFirst November 7, 2014; DOI: 10.1158/1535-7163.MCT-14-0542-T

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Molecular Cancer Therapeutics

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_mol Cancer Ther_ Published OnlineFirst November 7, 2014.

Updated version  Access the most recent version of this article at: doi:10.1158/1535-7163.MCT-14-0542-T

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