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Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Genetic Profiling to Determine Risk of Relapse-Free Survival in High-Risk Localized Prostate Cancer

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Abstract

Purpose: The characterization of actionable mutations in human tumors is a prerequisite for the development of individualized, targeted therapy. We examined the prevalence of potentially therapeutically actionable mutations in patients with high-risk clinically localized prostate cancer.

Experimental Design: Forty-eight samples of formalin-fixed paraffin-embedded prostatectomy tissue from a neoadjuvant chemotherapy trial were analyzed. DNA extracted from microdissected tumor was analyzed for 643 common solid tumor mutations in 53 genes using mass spectroscopy–based sequencing. In addition, PTEN loss and erythroblast transformation-specific–related gene (ERC) translocations were examined using immunohistochemistry (IHC) in associated tissue microarrays. Association with relapse during 5 years of follow-up was examined in exploratory analyses of the potential clinical relevance of the genetic alterations.

Results: Of the 40 tumors evaluable for mutations, 10% had point mutations in potentially actionable cancer genes. Of the 47 tumors evaluable for IHC, 36% had PTEN loss and 40% had ERG rearrangement. Individual mutations were not frequent enough to determine associations with relapse. Using Kaplan–Meier analysis with a log-rank test, the 16 patients who had PTEN loss had a significantly shorter median relapse-free survival, 19 versus 106 months ($P = 0.01$).

Conclusions: This study confirms that point mutations in the most common cancer regulatory genes in prostate cancer are rare. However, the PIK3CA/AKT pathway was mutated in 10% of our samples. Although point mutations alone did not have a statistically significant association with relapse, PTEN loss was associated with an increased relapse in high-risk prostate cancer treated with chemotherapy followed by surgery. *Clin Cancer Res; 1–7. ©2013 AACR.*

Introduction

A major focus of current clinical oncology is shifting from treating cancers based on organ of origin to treating cancers based on molecular characteristics of the tumor. The characterization of targetable genomic and molecular aberrations is a prerequisite to the development of successful targeted and individualized cancer therapies. In several tumor types, genetic and molecular alterations that are targets for therapy or guide selection of therapies have been reproducibly described, but to date this has not been consistently described in prostate cancer, the most common malignancy in men.

The vast majority of prostate cancers respond to targeting of the androgen signaling pathway, but most eventually regain the ability to proliferate despite therapeutic manipulation of androgen receptor (AR) signaling (1). Thus, new targets are needed to improve therapy for these castration-resistant prostate cancers. Multiple groups of researchers have described genomic alterations in prostate cancer (2–4). However, these have not yet been used as predictive biomarkers or as targets for personalized therapy. Many of the prior mutational studies in prostate cancer have investigated early stage, low-risk prostate cancers, or metastatic disease deposits. Our study uses cases of high-risk localized prostate cancers [selected as high risk based on clinical stage T2c or surgically resectable T3a, serum prostate-specific antigen (PSA) greater than or equal to 15 ng/mL, or a Gleason grade of at least 4+3 (i.e., 4+3, 4+4, or any 5 elements)] that were prospectively treated and collected as part of a study of neoadjuvant chemotherapy. Notably, there is a higher mutation rate in the PI3K (phosphoinositide 3-kinase)/AKT pathway in our series than has previously been published.

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Translational Relevance

Aside from the androgen signaling pathway, clinically relevant targets for prostate cancer therapy are needed. In this study, we used samples from high-risk prostate cancers that were followed prospectively and treated uniformly. We examined the prevalence of potentially actionable genetic abnormalities in these prostate cancers and correlated the findings with long-term clinical data. Prior studies have examined the genetic and molecular abnormalities in prostate cancer. This study adds more support for the clinical relevance of these abnormalities.

The PI3K/AKT pathway is one of the most commonly altered signaling pathways in prostate cancer. PTEN loss and upregulation of the AKT pathway have begun to emerge as potentially important aberrations in prostate cancer biology (5, 6). Abnormalities of this pathway have been shown to induce proliferation in multiple cancers, including prostate cancer (7). The loss of PTEN, a tumor suppressor gene that regulates the AKT pathway through negative feedback mechanisms (8, 9), has been shown to be associated with a more aggressive prostate cancer phenotype in both mouse models, and in human prostate cancer tumor interrogations (10–14).

Fusion of the *TMPRSS2* and *ERG* genes is the most common genetic translocation in prostate cancer and is seen in approximately 50% of human prostate cancer specimens (15). However, there has not been a consistent link between *TMPRSS2–ERG* fusion and prostate cancer progression or aggressiveness (16–18).

Our study combines the investigation of potentially targetable point mutations in multiple cancer growth pathways, in addition to PTEN loss and *TMPRSS–ERG* fusion, in localized prostate cancer and correlates this genetic and molecular information with prostate cancer biochemical relapse after radical prostatectomy.

Materials and Methods

Patients

Forty-eight samples were used from a previously reported neoadjuvant chemotherapy trial with institutional review board approval (19). This study includes all evaluable prostate cancer specimens from this neoadjuvant trial. The original neoadjuvant chemotherapy trial involved patients with high-risk prostate cancer defined as clinical stage T2c or surgically resectable T3a, serum PSA greater than or equal to 15 ng/mL, or a Gleason grade of at least 4+3 (i.e., 4+3, 4+4, or any 5 elements). Patients were recruited from four sites in the Pacific Northwest. Patients were treated with docetaxel and escalating doses of mitoxantrone for 16 weeks before prostatectomy. Biochemical relapse was defined as a PSA greater than or equal to 0.4 ng/mL after prostatectomy or the initiation of another cancer-directed therapy for any detectable PSA, which was the case in 1 patient. The patients were followed until they reached the PSA endpoint of greater than or equal to 0.4 ng/mL.

There was 1 patient lost to follow-up at 59 months that had not yet relapsed, with the longest follow-up without biochemical relapse being 127 months. Two subjects, one who died of lung cancer at 3 months after starting the study, and another who had adjuvant X-ray therapy at 2 months (before PSA being obtained), were censored. Patient characteristics are listed in Table 1.

Table 1. Clinical characteristics of the study population

Patient characteristics	
Age, y	
Median	63
PSA, ng/mL	
Median	12.2
	Total (%)
Gleason score	
≤6	8 (16.7)
7	20 (41.7)
≥8	20 (41.7)
Stage	
T2	23 (47.9)
T3	23 (47.9)
T4	2 (4.2)
LN status	
Positive	9 (18.8)
Negative	39 (81.3)

Samples

Prostatectomy specimens were preserved in formalin-fixed paraffin-embedded tissue. Prostatectomy specimens were obtained from patients at four institutions between January 2001 and November 2004. Samples from the radical prostatectomy specimens were used to create tissue microarrays (TMA) using pathologist identified tumor-rich areas of the prostatectomy specimen.

Genetic and molecular analysis

A genitourinary pathologist (C.L. Corless) reviewed the hematoxylin and eosin (H&E)-stained slides of the prostatectomy blocks and marked tumor-rich areas (at least 50% tumor cells) for manual dissection. Tumor was dissected from unstained slides by comparison with the pathologist-marked H&E stained slide. DNA was extracted using a QIAmp DNA minikit in accordance with the manufacturer's instructions. Seven hundred and fifty nanograms of DNA was used for the 53–gene MassARRAY platform (Sequenom MassArray). Full details of this mutation detection technique have been published previously (20). A list of genes interrogated can be found in Table 2 and a full list of mutations screened can be found in the Supplementary Appendix. PTEN was not examined for point mutations. Mutations identified by MassArray were confirmed either by

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Table 2. Genes analyzed using solid tumor mutation panel

Gene	Gene	Gene	Gene
AKT1	*FGFR1*	*KRAS*	*PIK3R4*
AKT2	*FGFR2*	*MAP2K1*	*PIK3R5*
AKT3	*FGFR3*	*MAP2K2*	*PKHD1*
ALK	*FGFR4*	*MAP2K7*	*PRKCB1*
BRAF	*FOXL2*	*MET*	*RAF1*
CDK4	*GNA11*	*MYC*	*RET*
CSF1R	*GNAQ*	*NEK9*	*SMO*
CTNNB1	*GNAS*	*NRAS*	*SOS1*
EGFR	*HRAS*	*NTRK1*	*STAT1*
ERBB2	*IDH1*	*NTRK2*	*TEC*
ERCC6	*IDH2*	*NTRK3*	*TP53*
FBX4	*IGF1R*	*PDGFRA*	
FBXW7	*KDR*	*PIK3CA*	
FES	*KIT*	*PIK3R1*	

NOTE: See the Supplementary Appendix for a detailed representation of mutations examined.

Sanger sequencing or by a semiconductor-based sequencing method (Ion Torrent; Life Technologies; ref. 21). Methods of DNA analysis on the Ion Torrent platform at our institution have been previously published (22).

PTEN loss was examined using TMAs prepared from the original prostatectomy specimens as previously described (13). We elected to use TMAs rather than total prostatectomy specimens for our immunohistochemical analyses to ensure tumor-rich areas were stained rather than a majority of admixed normal stroma. By allowing staining on a single slide, arrays also maximize the uniformity of staining across specimens. Multiple tumor cores from each prostatectomy specimen were examined. We used the Cell Signaling Technologies rabbit monoclonal D4.3 (Cat# 9188) for the PTEN staining. This antibody has been validated in prostate cancer TMAs in prior studies (13, 23, 24). This antibody performed consistently and reproducibly in our positive control cell lines and our sample prostate cancer panel (Supplementary Fig. S1). PTEN protein expression (detected by immunohistochemistry, IHC) has been shown to correlate highly with *PTEN* genomic loss (detected either through single-nucleotide polymorphism arrays or FISH) and was as sensitive for the detection of either heterozygous or homozygous deletion (13, 24). PTEN IHC was scored by a specialized genitourinary pathologist (G.V. Thomas). Scores were either 0 for no staining in the tumor, 1+ for intermediate staining, 2+ for strong staining. Staining was scored by intensity compared with adjacent benign glands and stroma, rather than by percentage of tumor involved as was the standard in previously reported studies (13). For statistical analysis, any staining (i.e., scores 1+ and 2+) was considered PTEN "positive" or expression of PTEN, and the absence of any staining (i.e., score 0) was considered as PTEN "negative" or PTEN loss (Fig. 1). IHC scoring was blinded with respect to clinical and molecular data, pathologic stage, Gleason score, and patient outcome.

TMPRSS–ERG translocation was investigated by IHC using a monoclonal antibody that binds to the truncated ERG in the fusion protein, as previously validated (25). We used the EPR3864 clone from Epitomics. TMAs from prostatectomy specimens were prepared and scored as follows: negative (0), weakly positive (1+), moderately positive (2+), strongly positive (3+). Endothelial cells and lymphocytes were used as internal controls. "Positive" staining indicated the presence of a *TMPRSS–ERG* translocation. For statistical analysis, any staining was considered to represent the presence of the *TMPRSS–ERG* translocation, and "negative" represented no protein expression, or no translocation present (Fig. 1).

Statistical analysis

The Fisher exact test was performed to assess association between prostate cancer relapse (categorized relapse time) and genetic and clinical covariates. The primary endpoint was biochemical relapse-free survival defined as the interval between date of surgery and the date of PSA relapse. Survival analysis was conducted to correlate genetic and clinical covariates with time to prostate cancer biochemical relapse using the Kaplan–Meier method, the log-rank test, and the Cox proportional hazard regression method. Median time to biochemical relapse and HRs with their 95% confidence interval (CI) were calculated. Statistical significance was defined as a P value of <0.05. All the statistical analysis was performed using SAS Version 9.3.

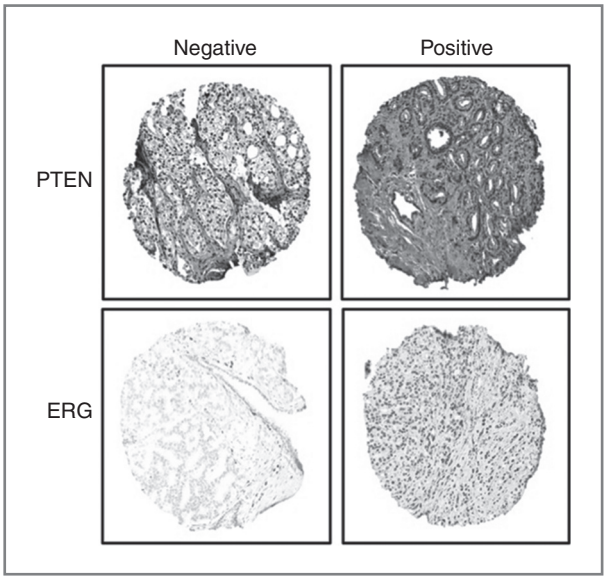

Figure 1. PTEN and ERG IHC from representative prostatectomy samples. PTEN and ERG scoring criteria defined: absence of protein expression by IHC for PTEN or ERG (i.e., score 0) was considered negative. Presence of staining (i.e., score +1 or +2 for PTEN; score +1 or +2 or +3 for ERG) was considered positive. Please see Materials and Methods for detailed description.

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Table 3. Median time to biochemical relapse

Variable	Total (%)	Median time to biochemical relapse (months)	*P* value[a]
Mutation			
Yes	4 (10)	75	0.80
No	36 (90)	31	
ERG			
Positive (translocation)	16 (34.8)	50	0.66
Heterogeneity	2 (4.4)	51	
Negative	30 (60.8)	47	
PTEN			
Negative (loss)	10 (21.3)	19	0.01
Heterogeneity	6 (12.8)	9	
Positive	31 (66)	106	
Gleason score			
≤6	8 (16.7)	54	0.88
7	20 (41.7)	51	
≥8	20 (41.7)	28	
Tumor stage			
T2	23 (47.9)	89	0.06
T3	23 (47.9)	19	
T4	2 (4.2)	N/A	
LN status			
Positive	9 (18.8)	6	<0.0001
Negative	39 (81.3)	75	

[a]Log-rank test.

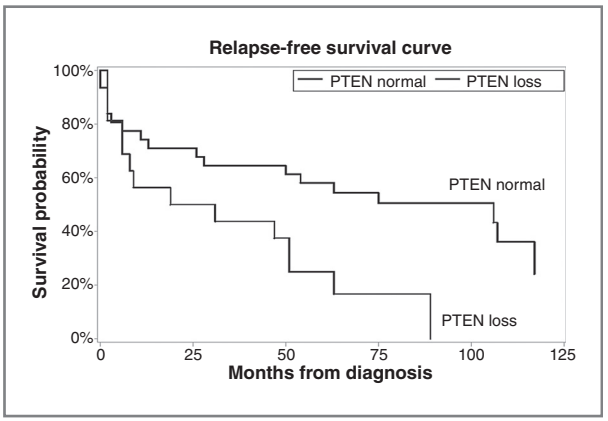

Figure 2. PTEN loss was associated with an increased risk of biochemical relapse with a median time to relapse of 19 months in the PTEN loss patients and 106 months in the PTEN normal patients ($P = 0.01$) with an HR of 2.47 for relapse in the PTEN loss group.

Results

There were 40 tumors that were evaluable for point mutations. Eight specimens did not have sufficient tumor in the specimen to yield DNA sufficient for mutational analysis on the MassArray system due to the large amount of normal stromal tissue admixed in the specimens. Tumor-rich areas of the prostatectomies, with at least 50% tumor cells, were used for analysis. All 48 specimens were evaluable for IHC for ERG and 47 were evaluable for PTEN.

Thirty-five percent of the samples showed *TMPRSS2–ERG* translocation by IHC (Table 3). Another 4% of samples showed marked heterogeneity in the ERG fusion protein in different TMA cores. Heterogeneity of ERG protein expression has been reported within the same tumor focus (i.e., intrafocal heterogeneity), both within TMA cores as well as whole sections in prior studies (26–29). Twenty-one percent of the samples showed PTEN loss, with an additional 13% showing marked heterogeneity, in which some tumor cores had no PTEN expression and some, from the same prostatectomy specimen, had strong staining for PTEN. PTEN heterogeneity in prostate tumors has been described in prior studies (13, 30). The rate of heterogeneity in our PTEN samples is in keeping with reported rates of 9% and 11% seen in prior TMAs and whole sections respectively, using the same antibody (13).

TMPRSS–ERG translocation was not associated with time to biochemical relapse (Table 3). PTEN loss, however, was associated with an increased risk of biochemical relapse with a median time to relapse of 19 months in the PTEN loss patients, 9 months in the PTEN heterogeneous patients, and 106 months in the PTEN normal patients ($P = 0.01$). PTEN loss was statistically associated with biochemical relapse independent of stage or grade. The HR was 2.47 (95% CI, 1.18–5.21) for biochemical relapse in the PTEN loss group (Fig. 2 and Table 4). Interestingly, in 1 patient who had evaluable lymph nodes, there was retained PTEN in the primary tumor, but complete loss of PTEN expression in the lymph node.

We used a prior validated solid tumor mutation panel (20) targeting 643 mutations in 53 genes (Table 2). We found four point mutations in the PI3K/AKT pathway; two cases had an *AKT* E17K mutation, one case with *PIK3CA* Q546P, and one with *PIK3CA* H1047R mutation. This

Table 4. Unadjusted HR for biochemical relapse

Characteristic	Unadjusted HR	95% CI	*P* value
Mutation			
Yes	0.86	0.26–2.87	0.80
No	Reference		
ERG			
Positive (translocation)	1.22	0.59–2.50	0.58
Negative	Reference		
PTEN			
Negative (loss)	2.47	1.18–5.21	**0.02**
Positive	Reference		

NOTE: Boldface indicates statistical significance.

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equates to an approximate 10% mutation rate in this pathway. There was no statistically significant association between these mutations and prostate cancer biochemical relapse, or other variables studied, likely due to the small number of cases. We did not find any other oncogenic mutations in other pathways represented on the panel. Although there is only a single example, the patient with both a point mutation in *PIK3CA* and complete loss of PTEN relapsed the earliest of the 4 patients with *AKT* or *PIK3CA* mutations. The other three samples with point mutations did not have concurrent loss of PTEN.

Discussion

The identification of uncommon but potentially actionable mutations in human tumors has the potential to change therapy and outcomes for a proportion of individuals with cancer. We examined a large number of potentially actionable mutations across 53 cancer-associated genes in prostatectomy specimens from men with newly diagnosed high-risk prostate cancer (based on Gleason score, PSA, or stage at diagnosis). We combined the mutational analysis with IHC techniques to discover PTEN loss and ERG translocations.

We found that approximately 10% of our prostate cancers had mutations in the PI3K/AKT pathway, which is slightly higher than in prior published reports which ranges from 1% to 5% (2, 4, 31–33). This may be because we screened for more mutations in this pathway, or perhaps because the higher-risk tumors that were selected for this analysis are more likely to have these mutations. Other studies have shown a 30% to 40% alteration rate in the PI3K/AKT pathway, but this includes changes in gene expression as well as mutation data, with only 1% to 2% point mutations detected in this pathway (4). Overall, approximately 40% of our samples had an alteration in the PI3K/AKT pathway, either activating point mutations or PTEN loss by IHC. It is interesting that this was the only pathway in which we identified mutations. No mutations were found in the mitogen-activated protein kinase pathway, including a number of receptor tyrosine kinases, the *RAS* genes, *BRAF*, and *MAP–ERK* kinase. There are other reports of similar mutation rates in the PI3K/AKT pathway, but mostly in metastatic disease (4), and in these studies there was no gene or pathway that was consistently mutated outside of the androgen signaling pathway. Increasingly, the PI3K/AKT pathway is believed to be an important nonandrogen signaling pathway in prostate cancer (12, 34, 35). We did not have the power to determine whether mutations in the PI3K/AKT pathway are associated with biochemical relapse-free survival.

We did, however, find a statistically significant association with biochemical relapse and PTEN loss by IHC. PTEN negatively regulates the PI3K/AKT pathway and loss of PTEN is associated with increased activity in the PI3K/AKT pathway and thus increased growth of the tumor cell (9). PTEN loss has been shown to decrease AR expression, potentially rendering tumors less sensitive to manipulation of the androgen signaling pathway (11, 35). However, a recent study showed that pharmacologic inhibition of the PI3K/AKT pathway activates AR signaling in PTEN-deficient tumors and, conversely, inhibition of AR promotes activity in the PI3K/AKT pathway in PTEN-deficient tumors (35). Such observations point toward opportunities to examine the clinical utility of simultaneously inhibiting PI3K/AKT pathway and AR signaling pathways in PTEN null tumors.

Our one sample with both a point mutation in PIK3CA and loss of PTEN had a highly aggressive Gleason 5+5 cancer with early biochemical relapse compared with other patients with only a PIK3CA or AKT mutation. This, in theory, could be due to increased activation of the PI3K/AKT pathway via an activating mutation along with removal of the negative regulation of this pathway with loss of PTEN. There are data that support the hypothesis that activation of PI3K/AKT pathway may be required for tumor growth in PTEN null tumors in cell culture and mouse models (34, 36).

Our study examined genetic alterations in early prostate cancers. However, there is evidence that there are genetic alterations that may not happen until late in the process of prostate cancer development. For instance, a recent study evaluated 45 prostatectomy and metastatic specimens and found genomic alterations in *BRCA* and *ATM* that only appeared in the castration-resistant prostate cancers (32). Such findings have reinforced our appreciation of the heterogeneity of solid tumors, even within individuals. In our localized prostate cancer specimens, we found a number of specimens that were heterogeneous for PTEN loss and TMPRSS–ERG translocation in the prostate alone. We do not have data from metastatic deposits that subsequently developed in some of our patients that would enable us to determine whether such heterogeneity is manifest in metastatic cancer.

Although it is theoretically possible that prior treatment with docetaxel and mitoxantrone could have played a role in selecting for tumors with these biologic features, it is unlikely that four cycles of chemotherapy would alter the genetic make-up of prostate cancer enough to either induce the genetic mutations, or induce *de novo* PTEN loss. It is more likely that pretreatment with chemotherapy would have enriched the specimens with more aggressive or resilient cancer cells. Having said that, the pathologic and clinical effects of chemotherapy on clinically localized prostate cancer are modest; thus, we do not expect that our observations were substantially shaped by this treatment exposure.

Technology that enables genomic analysis of tumors is evolving rapidly. The mass spectroscopy–based screening method used in our study can only identify hotspot mutations. In addition, our panel was focused on mutations known to activate oncogenes. The use of next-generation sequencing could perhaps find even more potentially targetable mutations. Nevertheless, our analysis provides important information with regard to a frequency of activation mutations of large number of potentially targetable genes in high-risk prostate cancer.

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This study adds to the growing evidence that the PI3K/AKT pathway may be a significant additional growth pathway in both hormone sensitive and castration-resistant prostate cancers. Our study is a unique contribution to the existing mutational data for multiple reasons. Our data are derived from samples in a selected high-risk localized prostate cancer population that was followed prospectively and treated uniformly. Many of the studies to date used multiple different stages of prostate cancers and these patients are not prospectively followed. In addition, we have a long follow-up to enable detection of late biochemical relapse to aid in a comprehensive understanding of the role of these genetic factors in the progression of prostate cancer. Our genetic analysis was a fairly comprehensive investigation of multiple mutations in numerous established oncogenes. This potentially enabled our higher detection of a 10% mutation rate in this the PI3K/AKT pathway.

In summary, we found that abnormalities of the PI3K/AKT pathway are the most common potentially targetable pathway in high-risk, localized prostate cancer. Notably, PTEN loss was significantly associated with an increased risk of biochemical relapse. Our results suggest that targeting this pathway may improve outcomes in selected patients with high-risk localized disease.

Disclosure of Potential Conflicts of Interest

M.C. Heinrich has honoraria from the speakers bureau of Novartis, received a commercial research grant from Novartis, has ownership interests in MolecualrMD, and is a consultant or advisory board member of MolecularMD, Ariad, and Novartis. C.L. Corless has honoraria from the Speakers Bureau of Novartis and Ion Torrent, and is a consultant or advisory board member of Novartis. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: M.C. Heinrich, C.S. Higano, M. Garzotto, T.M. Beer

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.M. Barnett, M.C. Heinrich, D. Nelson, T. Neff, C.S. Higano, M. Garzotto, C.L. Corless, T.M. Beer

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.M. Barnett, M.C. Heinrich, J. Lim, A. Warrick, T. Neff, C.S. Higano, M. Garzotto, G.V. Thomas

Writing, review, and/or revision of the manuscript: C.M. Barnett, M.C. Heinrich, J. Lim, C.S. Higano, M. Garzotto, D. Qian, G.V. Thomas, T.M. Beer

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.M. Barnett, M.C. Heinrich, D. Nelson, G.V. Thomas, T.M. Beer

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