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14. ABSTRACT

The goal of this project is to develop a multi-source biomarker panel based on blood obtained prior to surgery and tumor tissue from men undergoing radical prostatectomy for clinically low risk prostate cancer with known pathologic outcome information. In aim 1, the marker panel will be assessed for its ability to predict upgrading and upstaging between biopsy and pathology at prostatectomy. In Aim 2 we will assess the performance of a multi-source biomarker panel derived from blood, urine, and tissue among men accrued to an existing active surveillance cohort. In this aim, the marker panel will be tested for prediction of progression, specifically the extent to which the panel can add independent prognostic information to standard clinical variables. We have made progress toward the stated goals of the project over the past year. We have completed accession and processing of = 397 blood specimens from UCSF (Aim 1). Tissue analyses are ongoing for DNA and RNA assessments for UCSF patients. We are negotiating a regulatory issue for the Univ. of Washington specimens. Substantial quality of life surveys have been processed for Aim 2, and will be included in the multivariate analyses with the biomarkers. We look forward to completing our analyses and reporting results at the next year.

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Introduction

Identification of new biomarkers that more accurately distinguish indolent from aggressive low-risk prostate cancers would have a major impact on prostate cancer management. Patients with occult aggressive disease could be counseled appropriately for immediate treatment, while those with confirmed indolent disease could select and remain on surveillance with more confidence, and likely with a lesser burden of follow-up testing. Our aims are to validate, in both a pair of radical prostatectomy cohorts and in a multicenter active surveillance cohort, a set of urine, blood, and tissue-based biomarkers with respect to their prognostic utility.

Body

Task 1: Blood and tissue organization for Aim 1

We completed accession and processing of both blood and tissue specimens from both UCSF and UW. As in a prior progress report, the marginal cost for additional ELISA wells is negligible, so we began with N=397 available plasma specimens, i.e., 97 additional specimens beyond the original specified case-control study. We also received and processed plasma on 260 patients from UW, for a total N=657 for plasma analysis. All patients were diagnosed in 2000 or later with low risk disease (diagnosis PSA < 10 ng/ml, clinical stage T1-2, biopsy Gleason grade 2-6) and underwent radical prostatectomy monotherapy within 6 months. We completed pathology review and tissue punching required for tissue identification, re-reading, and punching cases on N=381 cases from UCSF and N=260 cases from UW, for a total N=641 for tissue analysis. Table 1 summarizes baseline clinical characteristics from these two cohorts:

Table 1: Baseline characteristics of UCSF and UW prostatectomy cohorts

Patient characteristics	Value	UCSF N=381	UW N=260	p-value
Age at diagnosis, mean (SD)	Years	58.5 (6.81)	59.1 (7.05)	[+]0.29
Race/ethnicity, n (%)	Asian	12 (3)	2 (1)	*<.01
	African American	10 (3)	1 (1)	
	Caucasian	340 (89)	174 (98)	
	Mixed	7 (2)	0 (0)	
	Other/unknown	12 (3)	0 (0)	
	Missing		*83*	
PSA at diagnosis, median (IQR)	Ng/ml	5.2 (4.2, 6.5)	4.7 (3.9, 6.1)	**<.01
Biopsy Gleason grade at diagnosis, n (%)	2-6	381 (100)	260 (100)	--

Patient characteristics	Value	UCSF N=381	UW N=260	p-value
Biopsy cores % positive at diagnosis, median (IQR)		25 (13, 42)	25 (14.5, 33)	**0.10
Clinical T-stage at diagnosis, n (%)	T1	227 (60)	202 (78)	*<.01
	T2	154 (40)	58 (22)	
Clinical CAPRA risk at diagnosis, n (%)	Low (0-2)	346 (91)	.	--
	Intermediate (3-5)	35 (9)	.	
	Missing		*260*	

UCSF, University of California San Francisco; UW, University of Washington; SD, standard deviation; IQL, interquartile range; CAPRA, UCSF Cancer of the Prostate Risk Assessment.

+p-value from Student's t-test; *p-value from Pearson chi-square;** p-value from Wilcoxon test

This was a case-control design, with the primary endpoint being increase in grade or stage to GS ≥3+4 and/or pathologic stage ≥T3a between biopsy and prostatectomy. A prespecified subset analysis looked at cases with "major" upgrading/upstaging to Gleason ≥4+3 or pathologic stage ≥T3b, respectively. The outcomes are summarized in Table 2:

Table 2: Upgrading/upstaging outcomes for Aim 1

Outcomes	Value	UCSF N	(%)	UW N	(%)	Chisq p-value
Any upgrade/upstage at RP	No	202	53	155	56	0.52
	Yes	179	47	124	44	.
Major upgrade/upstage at RP	No	336	88	257	92	0.10
	Yes	45	12	22	8	.
Outcome group	No change	202	53	155	56	0.26
	Minor increase	134	35	102	37	.
	Major increase	45	12	22	8	.

Over the course of our analyses for this project and other work we are doing in parallel, it has become clear that the upgrading/upstaging endpoint is imperfect, particularly because many cases with minimal representation of Gleason pattern 4 are "upgraded" but likely are biologically indistinguishable from non-upgraded cases. Likewise, defining "major" upgrade is somewhat problematic because the difference between Gleason 3+4 and Gleason 4+3—i.e., greater or less than 50% pattern 4—is again arbitrary and may not capture biology adequately [see an unrelated paper we published during the study

period on quantifying Gleason score: Reese et al. Cancer 2012; 118:6046]. Therefore we elected to modify our analysis plan based on other recent experience with another biomarker validation effort [see Klein, Cooperberg et al. Eur Urol 2014; 66:550], and set up the primary analysis as a multinomial regression allowing gradations of both upgrading and upstaging to be considered simultaneously. The 9 cells of the regression matrix are as follows:

pGS	pTstage T2	pTstage T3a	pTstage T3b
3+3	A	B	C
3+4	D	E	F
≥ 4+3	G	H	I

In this framework, cell A denotes controls, B-I any upgrade/upstage, BDE a minor upgrade/upstage, and DFGHI a major upgrade and/or upstage.

All cases retrieved were successfully cut and sent to the Paris lab and to Myriad genetics for DNA and RNA extraction, respectively.

Task 2: Blood and urine organization for Aim 2

The total enrollment to the Prostate Active Surveillance Study (PASS) is now over 1300. All of these men have contributed baseline urine and serum specimens. Median follow-up at this point is 3.3 years from diagnosis. Over 287 men have progressed by study criteria. We increased the N for this project to the first 500 men enrolled. Of these, 145 (29%) had any reclassification by either Gleason grade or tumor volume criteria: 89 (61% of those reclassified) by grade alone, 18 (12%) by volume alone, and 38 (26%) by both criteria. 87 of these men (19%) have gone on to prostatectomy and have surgical pathology results available. The median followup is 4.0 years from diagnosis and 3.0 years from enrollment. Table 3 summarized the baseline demographic and clinical characteristics for this cohort.

Table 3: Baseline characteristics for PASS cohort

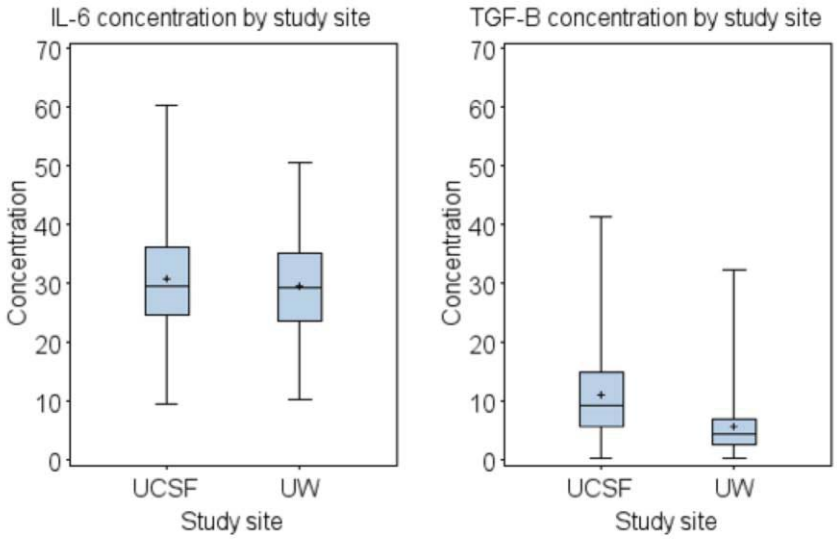
Patient Characteristic	N, %
Race	
Caucasian	457 (92)
African American	17 (3)
Asian	18 (4)
Other	4 (1)
Unknown	4
Ethnicity (Latino/Hispanic)	
Yes	21 (5)
No	472 (95)
Unknown	7
Age	
<50	22 (4)
50-60	141 (28)

61-70	258 (52)
>70	79 (16)
PSA at entry	
0 – 3.99	205 (41)
4.0 – 10.0	266 (53)
>10.0	29 (6)
Clinical T-stage	
T1	436 (87)
T2a	61 (12)
T2b+ T2c	3 (1)
Gleason Score	
≤6	461 (92)
7 (3+4)	37 (7)
7(4+3)	2 (1)
Tumor Volume, % positive cores	
1 – 10	214 (53)
11 – 30	163 (40)
≥31	30 (7)
Unknown	93
PSA Density	
0 – 0.15	255 (74)
0.151- 0.30	76 (22)
> 0.30	12 (4)
Unknown	157

Task 3: Serum analyses (Aims 1 and 2)

We completed all TGFβ1 and IL6SR analyses on the N=397 UCSF Aim 1 specimens, N=260 UW specimens, (Table 1) and the N=505 PASS Aim 2 specimens. Given an unexpected finding of different mean scores between the two cohorts and the Canary (Aim 2) specimens, we repeated the UCSF analyses during the previous year. The repeat analyses are more consistent with the other cohorts, and likely represent batch differences in the ELISA plates used. Normalization of TGFβ1 levels for PF4 levels as described in an early progress report had no substantive effect on the results, so these analyses were performed unadjusted. Box plots of plasma concentrations of both markers are illustrated in Figure 1, indicating a possible cohort effect for TGFβ1 but not IL6-SR.

Figure 1: IL6-SR and TGFβ1 levels in the UCSF (N=397) and UW (N=260) cohorts



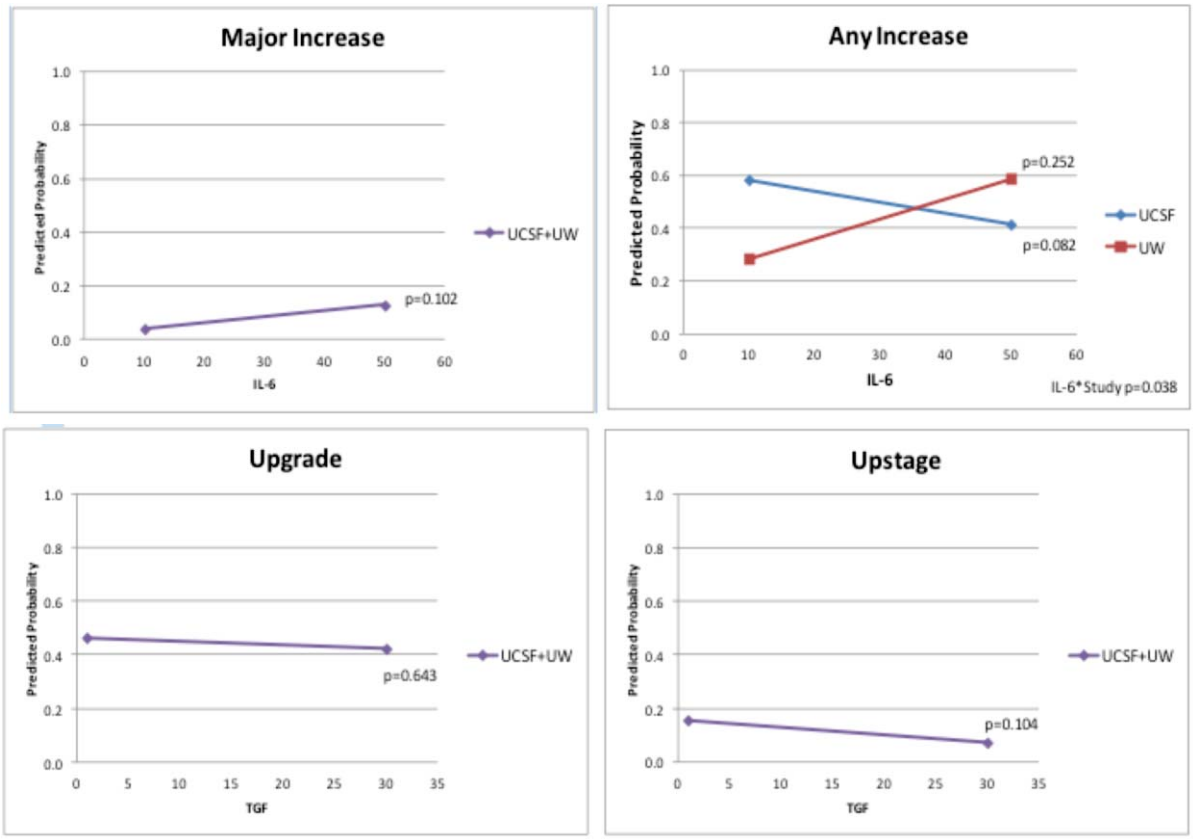
Each plasma biomarker was analyzed in logistic regression models adjusting for age, percent biopsy cores positive, PSA, and study site. Despite re-processing the UCSF specimens as described above, we found unexpected statistical interaction between IL-6SR and study site (UCSF vs. UW). On multivariable analysis, IL-6SR associated with upgrading/upstaging in opposite directions between the two cohorts, though neither was statistically significant (Table 4, Figure 2).

Table 4: Logistic regression results for IL-6SR and for TGFβ1. Age, % cores positive, and PSA predicted the primary outcome (any increase in grade) but the markers did not. Only age predicted major increase in grade/stage.

	MAJOR INCREASE (69 events)				ANY INCREASE (310 events)			
EFFECT	P-VAL	OR	95%CI LL	95%CI UL	P-VAL	OR	95%CI LL	95%CI UL
Main								
Age (years)	<.0001	1.091	1.048	1.135	<.0001	1.056	1.031	1.082
Cores % positive	0.9904	1.000	0.987	1.013	0.0004	1.015	1.007	1.023
PSA (ng/ml)	0.2082	1.093	0.952	1.256	0.026	1.103	1.012	1.202
Study UW v UC	0.1946	0.695	0.401	1.204	0.0437	1.107	0.798	1.537
IL-6 (log_il6 at both sites)	0.1022	2.158	0.858	5.429	*N/A (interaction significant)*			
Interaction	*N/A (interaction not significant)*							
*IL-6*Study*					*0.0383*	-	-	-
UW-specific*								
IL-6 (log_il6 at study=UW)					0.2522	2.211	0.905	5.398
UC-specific*								
IL-6 (log_il6 at study=UC)					0.0816	0.654	0.316	1.353

	MAJOR INCREASE (69 events)				ANY INCREASE (310 events)			
EFFECT	P-VAL	OR	95%CI LL	95%CI UL	P-VAL	OR	95%CI LL	95%CI UL
Main								
Age (years)	<.0001	1.087	1.044	1.13	<.0001	1.055	1.03	1.08
Cores % positive	0.9365	0.999	0.987	1.012	0.0005	1.014	1.006	1.023
PSA (ng/ml)	0.1923	1.095	0.955	1.255	0.0208	1.107	1.016	1.206
Study UW v UC	0.2606	0.712	0.394	1.287	0.8148	1.044	0.728	1.496
TGF (log_tgfb at both sites)	0.5703	1.102	0.789	1.538	0.6112	0.948	0.771	1.165
Interaction	*N/A (interaction not significant)*				*N/A (interaction not significant)*			
*TGF*Study*								
UW-specific*								
TGF (log_tgfb at study=UW)								
UC-specific*								
TGF (log_tgfb at study=UC)								

Figure 2: Plots of multivariable model predictions of any or major increase in grade/stage for IL-6 (top panels) and TGFβ1 (bottom panels).



Task 4

All N=500 PASS participants (Table 3) have had post-DRE urine specimens transferred to GenProbe for analysis of urinary PCA3 and TMPRSS2:ERG levels, all of which have now been processed (Table 5). Work preceding this project showed positive associations between the urinary markers and baseline tumor characteristics [Lin et al. Clin Cancer Res 2013; 19:2442].

Table 5: Distribution of plasma IL-6SR, plasma TGF1, urine PCA3, and urine T2-ERG levels in the PASS cohort

	N	Mean	Std.	Q1	Median	Q3	Q. range	Range
Diagnosis to Baseline (year)	500	1.35	1.49	0.38	0.71	1.88	1.50	8.63
IL6	500	47.95	16.68	35.52	45.58	54.99	19.47	148.65
TGF	500	3.74	3.64	1.69	2.57	4.29	2.60	30.69
logPCA3 score	500	3.43	0.95	2.83	3.41	4.04	1.21	8.78
logT2-ERG score	500	1.60	3.86	0.98	2.55	4.00	3.02	16.85

Figure 3: Kaplan-Meier plots of time to progression/reclassification in PASS statified by median score for plasma IL-6SR (top left), plasma TGF1 (top right), urine PCA3 (bottom left), and urine TMPRSS2:ERG (bottom right)

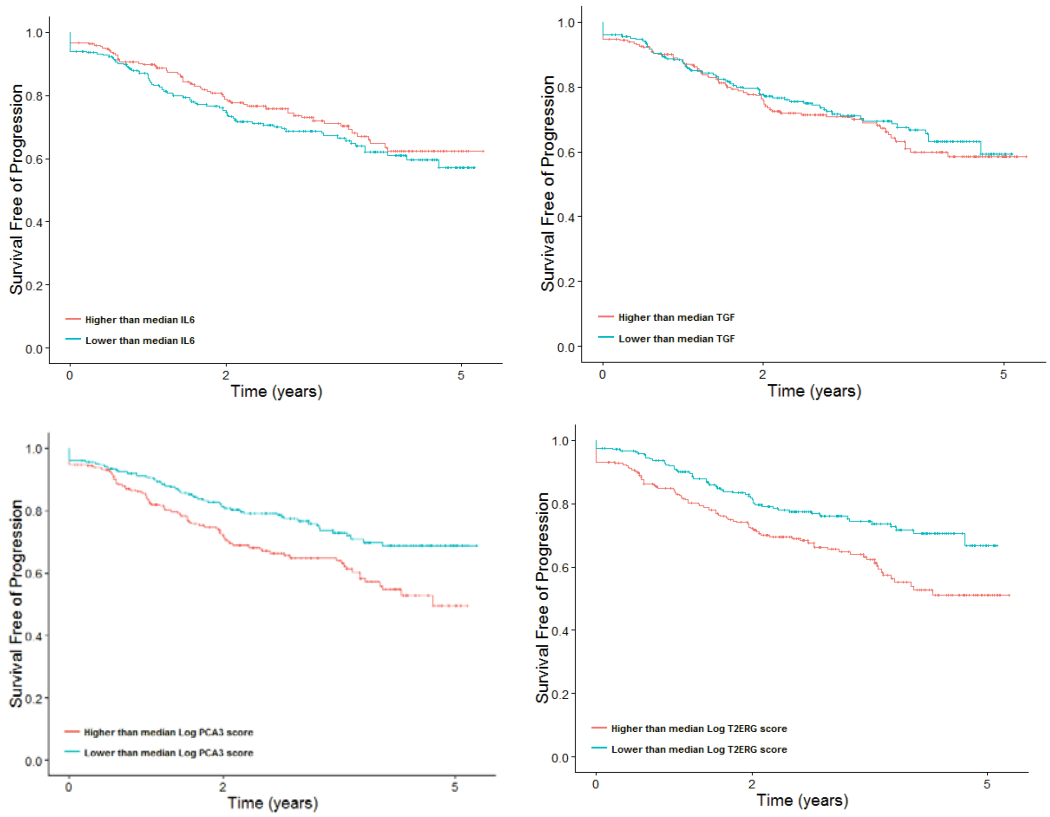


Table 6: Cox survival analysis in PASS cohort: time to progression / reclassification

Patient Characteristic	N	Unadjusted Haz. Ratio (95% CI)	N	Adjusted Haz. Ratio (95% CI)
Race				
Caucasian	457	Ref	211	Ref
African American	17	1.28 (0.52, 3.13)	9	1.24 (0.35, 4.42)
Asian	18	0.89 (0.36, 2.17)	7	0.39 (0.05, 3.01)
Other	8	2.71 (0.67, 10.98)	2	4.74 (0.59, 37.97)
Ethnicity (Latino/Hispanic)				
Yes	21	Ref	7	Ref
No	472	0.91 (0.40, 2.06)	222	0.43 (0.10, 1.86)
Age				
<50	22	Ref	7	Ref
50-60	141	1.00 (0.35, 2.80)	70	1.45 (0.18, 11.69)
61-70	258	1.22 (0.45, 3.34)	118	1.78 (0.22, 14.26)
>70	79	1.37 (0.48, 3.92)	34	2.37 (0.28, 19.86)
PSA at entry				
0 – 3.99	161	Ref	89	Ref
4.0 – 10.0	222	1.69 (1.16, 2.47)	128	0.66 (0.36, 1.23)

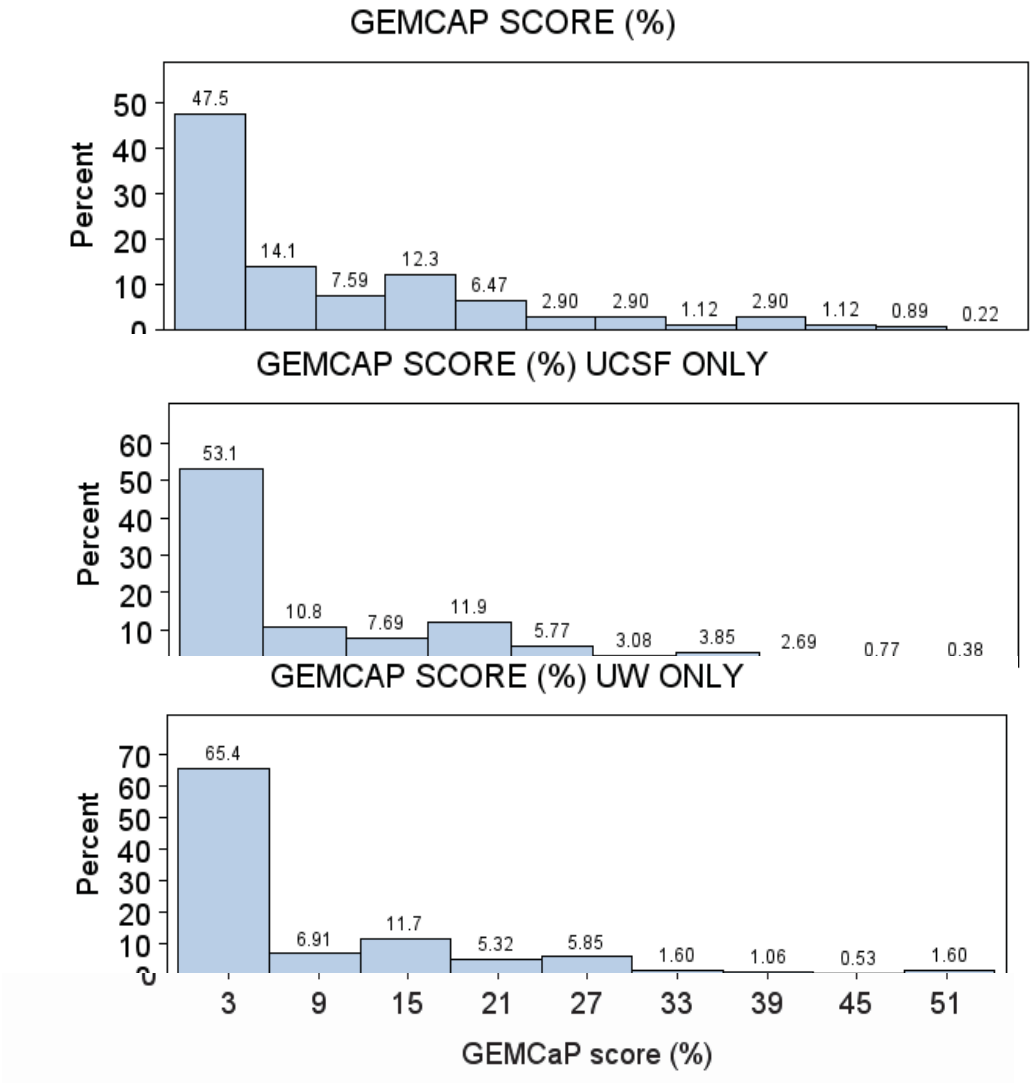
>10.0	23	0.58 (0.18, 1.86)	12	0 (0, -)
Clinical T-stage				
T1	436	Ref	197	Ref
T2a	61	0.95 (0.57, 1.60)	30	0.93 (0.44, 1.98)
T2b+ T2c	3	0 (0, -)	2	0 (0, -)
Gleason Score				
≤6	461	Ref	210	Ref
7 (3+4)	37	0.86 (0.45, 1.63)	18	0.45 (0.17, 1.20)
7(4+3)	2	0 (0, -)	1	0 (0, -)
Tumor Volume, % positive cores				
1 – 10	214	Ref	118	Ref
11 – 30	163	2.25 (1.53, 3.31)	93	2.65 (1.53, 4.56)
≥31	30	4.96 (2.76, 8.89)	18	8.23 (3.64, 18.57)
PSA Density				
0 – 0.15	200	Ref	158	Ref
0.151- 0.30	69	1.92 (1.23, 2.99)	62	2.49 (1.30, 4.75)
> 0.30	10	0.90 (0.22, 3.68)	9	2.22 (0.50, 9.86)
IL6	500	1.00 (0.99, 1.01)	229	0.99 (0.97, 1.01)
TGF	500	1.04 (1.00, 1.08)	229	1.05 (0.99, 1.12)
Log(PCA3 score)*	500	1.30 (1.09, 1.55)	229	0.97 (0.74, 1.28)
Log(T2 ERG Score)*	500	1.08 (1.02, 1.14)	229	1.03 (0.96, 1.11)

Task 5

As described above, N=674 total specimens have been cut and sent to the Paris lab and to Myriad Genetics. The Paris lab has completed DNA extraction and array comparative genomic hybridization (aCGH) analysis on the full sample set. Myriad likewise has completed RNA extraction and RT-PCR on the same cases, and we have GEMCaP and Prolaris (CCP) scores from the DNA and RNA, respectively.

In terms of GEMCaP score distribution, most cases were notably low-risk; in fact, nearly 50% had a score of 0, indicating no amplification of any of the loci previously associated with aggressive disease (Figure 4). Interestingly, the UW cohort was even lower risk on average than the UCSF cohort in terms of GEMCaP scores.

Figure 4: GEMCaP score distribution for the overall cohort (top panel), for UCSF cases (middle panel), and for UW cases (bottom panel)



GEMCaP scores were higher for men with upgrading/ upstaging on final pathology compared to those with no upgrading/upstaging, and those with major upgraded/ upstaged pathology had the highest scores (figure 5).

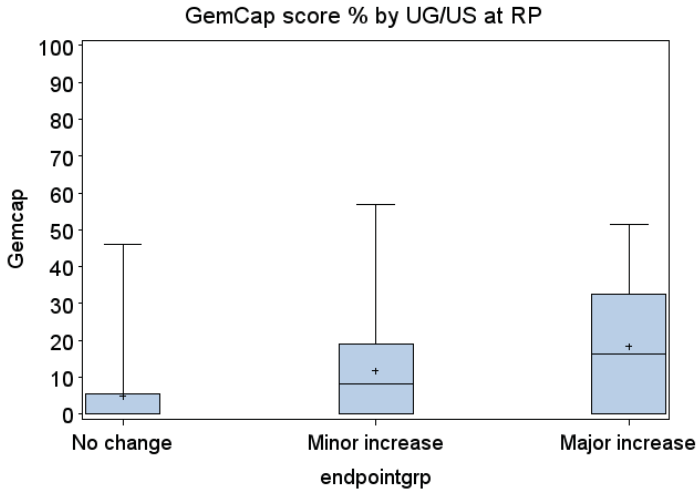


Figure 5: box plot of GEMCaP scores by final pathology

On the binomial multivariable analysis originally specified in the proposal, GEMCaP score statistically significantly associated with both any upgrade/upstage (OR 1.07, 95% CI 1.04-1.09) *and* with major upgrade/upstage (OR 1.05, 95% CI 1.02-1.07) after adjustment for age, % of cores positive, PSA at diagnosis, year of diagnosis, and cohort (UCSF vs. UW). Increasing age was also associated with increased risk of adverse pathology, and UW cases had a 1.4-fold higher risk of minor upgrade/upstage (p=0.12) and 2.5-fold higher risk of major upgrade/upstage (p=0.03) compared to UCSF cases, perhaps reflecting different practices in terms of Gleason grade assignment on biopsies between the two institutions, a phenomenon previously reported from our two institutions among others from PASS [McKenney et al. J Urol 2011; 186:465]. Outcomes were very similar on the multinomial analysis described above (table 7).

Table 7: Multinomial regression indicating independent prediction of both minor and major upgrading / upstaging by GEMCaP as a continuous variable.

Effect	**Outcome: UG/US at RP (ref=None)**	Global p	Parameter p	OR	95% LL	95% UL
Base GemCap score (%)	Major increase	<.01	<.01	1.088	1.053	1.124
	Minor increase	.	<.01	1.064	1.040	1.088
Age at diagnosis (years)	Major increase	0.02	<.01	1.097	1.025	1.173
	Minor increase	.	0.07	1.032	0.998	1.067
Diagnostic biopsy % positive cores	Major increase	0.19	0.76	0.996	0.973	1.020
	Minor increase	.	0.11	1.009	0.998	1.021
PSA at diagnosis (ng/ml)	Major increase	0.25	0.39	1.108	0.879	1.398
	Minor increase	.	0.11	1.103	0.979	1.244
Year of diagnosis	Major increase	0.07	0.06	0.794	0.622	1.013
	Minor increase	.	0.45	1.046	0.931	1.176
Study (UW vs UCSF)	Major increase	0.05	0.02	2.940	1.206	7.167
	Minor increase		0.26	1.290	0.827	2.013

The distribution of Prolaris CCP scores is illustrated below in Figure 6. Distributions of log-transformed scores were similar between the UCSF and UW cohorts.

Figure 6: GEMCaP score distribution for the overall cohort (top panel), for UCSF cases (middle panel), and for UW cases (bottom panel)

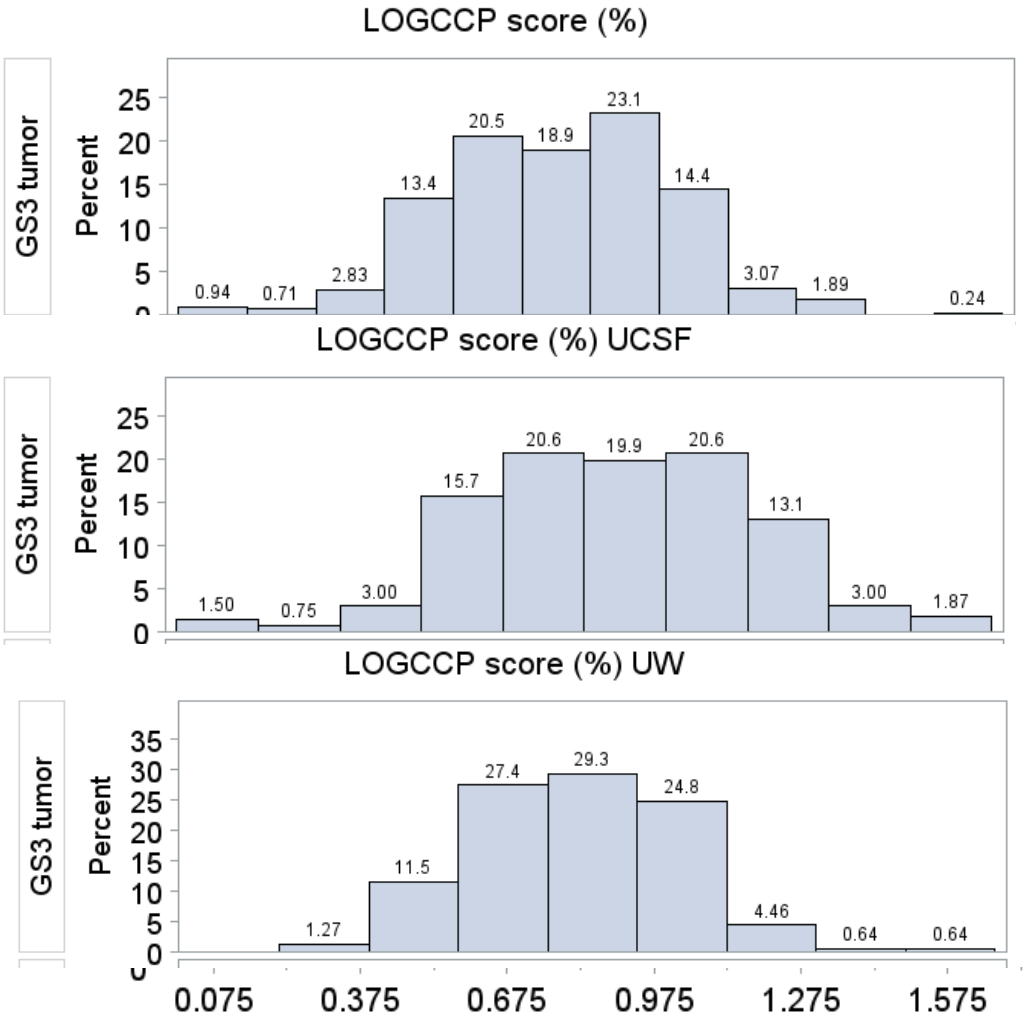


Figure 7 shows a boxplot of CCP scores by outcome. There were again differences in score distribution by outcome, though there was more overlap across groups than was seen with the GEMCaP score.

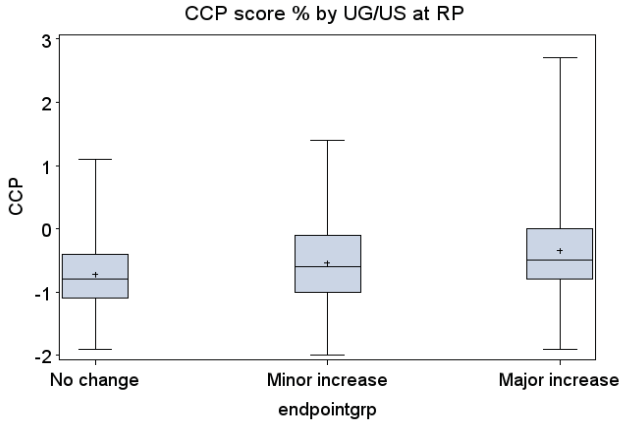


Figure 7: box plot of GEMCaP scores by final pathology

On binomial regression analyses, the CCP score was associated with minor upgrade/upstage (OR 4.2, 95% CI 1.7-10.3, p<0.01). The CCP also tended to predict major upgrading though this was not statistically significant (OR 4.4, 95% CI 0.3-23.0, p=0.08). There was no cohort effect observed to UCSF vs. UW as there was for GEMCaP. On the refined multinomial regression, CCP and PSA at diagnosis were the only statistically significant predictors of outcome (table 8).

Table 8: Multinomial regression indicating independent prediction of both minor and major upgrading / upstaging by CCP score as a continuous variable.

Effect	**Outcome: UG/US at RP (ref=None)**	Global p	Parameter p	OR	95% LL	95% UL
Base CCP score (log)	Major increase	<.01	0.02	8.486	1.488	48.405
	Minor increase	.	<.01	3.794	1.506	9.553
Age at diagnosis (years)	Major increase	0.05	0.12	1.055	0.986	1.129
	Minor increase	.	0.03	1.038	1.004	1.073
Diagnostic biopsy % positive cores	Major increase	0.12	0.21	0.983	0.957	1.010
	Minor increase	.	0.19	1.008	0.996	1.019
PSA at diagnosis (ng/ml)	Major increase	<.01	0.02	1.308	1.041	1.644
	Minor increase	.	<.01	1.181	1.044	1.336
Year of diagnosis	Major increase	0.20	0.14	0.843	0.671	1.060
	Minor increase	.	0.51	1.039	0.927	1.166
Study (UW vs UCSF)	Major increase	0.64	0.35	1.497	0.640	3.506
	Minor increase		0.92	1.024	0.649	1.615

With promising results for both of our tissue-based assays, we next examined the two in combination. First we examined correlation between the GEMCaP and CCP scores, since high correlation would suggest that the biological information represented by both is similar and would not both add independent value. This result is shown below in Figure 8: correlation was present but weak, and a great deal of

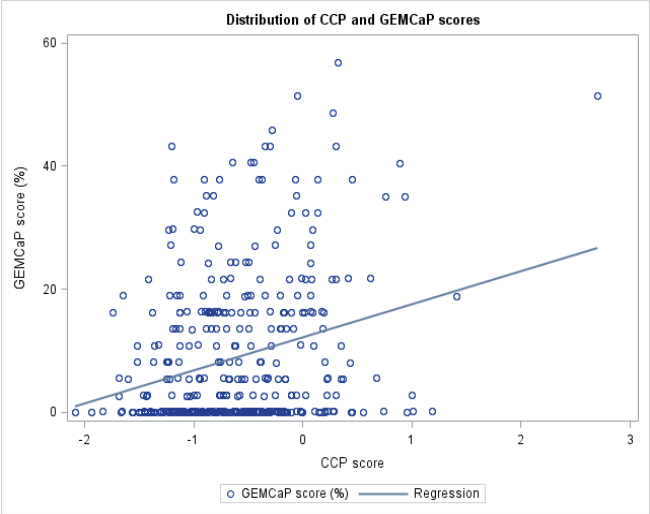


Figure 8: scatterplot of GEMCaP by CCP score, with linear regression line shown

scatter was present, suggesting potential for independent predictiveness.
We therefore ran a multivariable analysis including both tissue-based biomarkers. On binomial analysis, both CCP score and GEMCaP score predicted minor upgrading (p=.02 and p<0.01, respectively), adjusting for age, % of cores positive, PSA at diagnosis, year of diagnosis, and study cohort (none of which was a statistically significant predictor of outcome). GEMCaP but not CCP was a statistically significant predictor of major upgrading (p=0.02 and p=0.18, respectively).

On multinomial analysis, which we feel best reflects the spectrum of biology we are aiming to predict, both the CCP and GEMCaP scores *independently* predicted final surgical pathology, whereas none of the other parameters did so (table 9).

Table 9: Multinomial regression for prediction of final surgical pathology

Effect	**Outcome: UG/US at RP (ref=None)**	Global p	Parameter p	OR	95% LL	95% UL
Base CCP score	Major increase	0.04	0.04	2.263	1.045	4.901
	Minor increase	.	0.03	1.617	1.052	2.488
Base GEMCaP score	Major increase	<.01	<.01	1.074	1.036	1.114
	Minor increase		<.01	1.053	1.029	1.078
Age at diagnosis (years)	Major increase	0.14	0.26	1.043	0.969	1.122
	Minor increase	.	0.06	1.035	0.998	1.072
Diagnostic biopsy % positive cores	Major increase	0.11	0.15	0.977	0.946	1.009
	Minor increase	.	0.25	1.007	0.995	1.020
PSA at diagnosis (ng/ml)	Major increase	0.15	0.19	1.192	0.918	1.547
	Minor increase	.	0.08	1.121	0.984	1.277
Year of diagnosis	Major increase	0.19	0.13	0.815	0.626	1.061
	Minor increase	.	0.59	1.034	0.914	1.171
Study (UW vs UCSF)	Major increase	0.26	0.11	2.207	0.840	5.797
	Minor increase		0.45	1.204	0.746	1.945

These results suggest that alterations in DNA copy number and RNA expression are both predictively—and more importantly are *independently* predictive of outcomes, and that unique insights into tumor biology may be gleaned by analysis of both types of biomarkers.

Task 6

As noted previously, the VSIMS database has been updated to accommodate new tissue-based data and QOL fields, and biomarker data have been entered.

The results of the merged biomarker analysis have been presented throughout this report. In addition to the GEMCaP analyses described in the grant, we will further examine the fraction of the genome altered (FGA), based on the empiric observation that some of these low-risk cases have very low FGAs—perhaps indicative of the most indolent prostate tumors that could be managed with a low-intensity surveillance approach. Additionally, though not specified in the original grant application, we recognized the opportunity to perform heterogeneity studies in the cases which were in fact upgraded on final surgical pathology; for these cases we sampled both the Gleason 3 and Gleason 4 tumor areas and processed both for GEMCaP and Prolaris scores.

We have also received and completed initial processing on 1762 full quality of life surveys, of which 550 also include detailed diet and lifestyle information. Descriptive summary spaghetti plots for two example domains for urinary obstruction and sexual function (two domains expected to affect treatment decisions for men on surveillance) are included below as Figure 9.

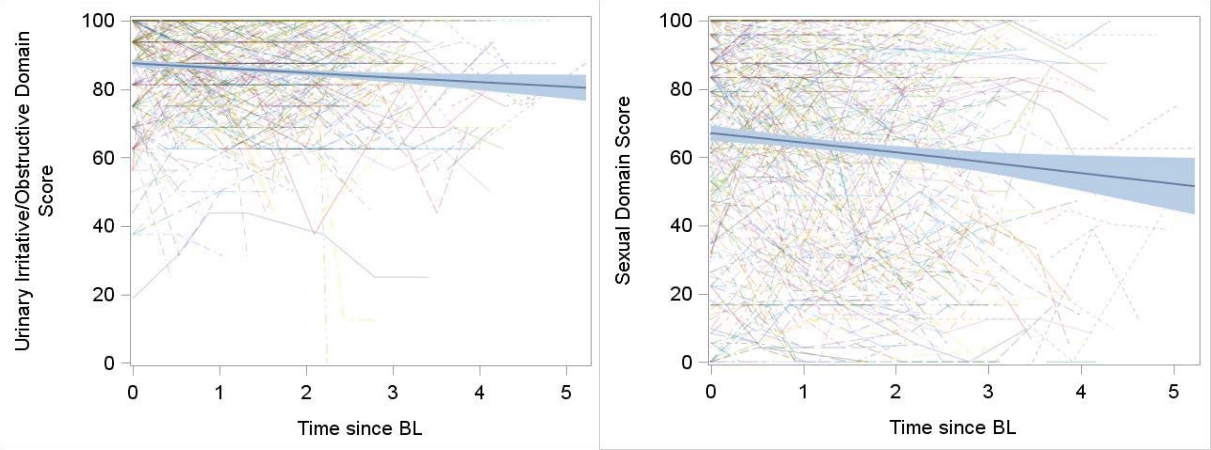


Figure 9: Spaghetti plot of urinary irritation/obstruction (left) and sexual function (right) over time after diagnosis of prostate cancer. The plots indicate gradual declines over time in both domains, as well as expected massive variation in sexual function both at baseline and in followup.

These data represent the most thorough and complete QOL assessment yet performed in an active surveillance cohort. We are continuing to collect further followup data and will be analyzing the effects of both baseline and followup QOL on treatment decision making for men embarking on AS as initial management.

Key Research Accomplishments

- Analysis of baseline urine specimens in PASS (Aim 2) for PCA3 and TMPRSS2:ERG indicated that both markers are associated with higher-volume prostate cancer and with the presence of high Gleason grade tumors at baseline. Both markers combined with PSA yielded better ROC curve results for prediction of high grade disease (AUC 0.70) than any of the markers alone. However, these markers do not necessarily appear to be *independently* associated with outcomes following diagnosis, as they associate closely with known clinical parameters including Gleason score.
- IL-6 and TGFβ1, markers previously associated with biochemical recurrence after prostatectomy for relatively high risk disease, do not appear to be statistically significantly predictive of either early endpoint analyzed—upgrading/upstaging from clinically low risk disease or early reclassification on active surveillance.
- On the other hand, both the CCP score based on RNA expression and the GEMCaP score based on DNA copy number variation appear independently predictive of adverse pathology at prostatectomy as assessed by both any and major upgrading/upstaging.
- We have also amassed the best extant dataset for QOL outcomes for men on active surveillance, which will serve as the basis for multiple future analyses.

Reportable Outcomes

1. A manuscript, "Urinary TMPRSS2:ERG and PCA3 in an Active Surveillance Cohort: Results from a Baseline Analysis in the Canary Prostate Active Surveillance Study" (Lin DW et al, Clin Cancer Res 2013; 19:2442) was published as noted previously.
2. A manuscript "Outcomes of active surveillance for the management of clinically localized prostate cancer in the prospective, multi-institutional Canary PASS cohort" updating results from the PASS cohort from a clinical standpoint has been published (Newcomb LF et al. Outcomes of Active Surveillance for Clinically Localized Prostate Cancer in the Prospective, Multi-Institutional Canary PASS Cohort. J Urol 2016; 193:313).
3. Building from our biomarker validation experience accumulating under this grant and elsewhere, we competed successfully for a 2012 DOD Transformative Impact Award PC121236 "Development, validation, and dissemination of an integrated risk prediction model and decision aid to discern aggressive versus indolent prostate cancer," which been awarded. Work is well underway, and we will have the opportunity to compare biomarker results directly in the two cohorts funded. There is substantial synergy between the two DOD grants both from infrastructure and scientific standpoints.

4. Dr. Cooperberg was recently awarded a revised NIH/NCI grant entitled, "Improving prostate cancer outcome prediction through noninvasive exRNA assessment," submitted in response to PA-13-302 Research Project Grant (Parent R01). This is his first R01 as PI. This proposal directly leverages the study populations and data/biospecimen resources being amassed as part of both Aims of this IMPACT award, and *will allow direct comparison of additional blood-based biomarkers based on analysis of plasma micro RNA (miRNA) together with the markers already underway with others and studies under this award*. The revised grant, R01 CA198145-01A1, funded as of August 2016, and we are getting underway with the project currently.

Conclusion

We have completed the plasma, urine, and tissue assays laid out in the project and described in detail above. While the plasma and urine tests examined did not prove independently predictive of outcomes, the tissue tests are both highly promising, and more intriguingly both appear to be *independently* of adverse pathology, suggesting potentially additive information. We are finalizing a few additional analyses, and anticipate at least two manuscripts from the results described here, one for the plasma and urine results and one for the tissue results. As noted above, the data generated but beyond scope of the grant itself will also allow us to report what we anticipate will be important novel findings regarding intra-prostatic heterogeneity, and additional detailed analysis of DNA copy number variation beyond the GEMCaP score itself.

Appropriately validating biomarkers, assessing their independent contribution to prognostic assessment, and determining their optimal clinical use and cost-effectiveness all require carefully designed analyses using well-described tissue repositories—exactly the sort of work we have completed under this grant. The tissue results are quite exciting, and while the urine and plasma results are disappointing, the project has directly laid the groundwork for further studies on these same specimens using next-generation "liquid biopsy" approaches which were not available when this grant was written—such as the miRNA project recently funded as noted above.

The field of prostate cancer biomarkers is very rapidly expanding, and this grant has helped us build the foundation on which we are now planning and executing many additional studies. This foundation has helped establish our research group as national leaders in biomarker validation, and we remain very grateful for the support of the DOD and the CDMRP.