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14. ABSTRACT Our objective is to create a multi-institutional tissue microarray resource from radical prostatectomy samples with detailed clinical information and follow-up and rigorous case-cohort design for use as a platform for validating tissue biomarkers of prognosis. In addition, we have proposed testing a series of biomarkers of prognosis and a set of biomarkers that correlate with Gleason Score. We have made significant progress over the past year. We have completed the tissue microarrays and finalized standard procedures for tissue microarray storage, sectioning and shipping. We have set up a structure for reviewing and approving biomarker proposals based on sound scientific principles and strong preliminary data. We have devised and tested a centralized distribution mechanism at Stanford University of collating and shipping TMAs to participating sites, We have found shortcomings with the BLISS system and STMAD for histological image capture and storage for pathological review and have developed a much improved, highly efficient system using a Leica scanner and Path.XL image analysis software suite. We also have made significant progress in testing TACOMA, an automater TMA scoring algorithm. We have completed staining of the TMAs for H & E, High Molecular Weight Keratin, p27, ERG, SPKINKI, Ki67 (MIBI), MUCI, Survivin and PTEN FISH. Over the next year, we will expand our database to add more tested TMAS Biomarkers, perform QA/QC to ensure high quality, and evaluate their performance for predicting recurrence. We will further refine TACOMA algorithm to facilitate the scoring of TMA stains. We will work with investigators to write papers reporting tested TMA Biomarkers.

15. SUBJECT TERMS-
TMA, Recurrence, biomarkers, Case-Cohort design, antibody

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Introduction

The most significant challenge in managing localized prostate cancer is the decision of whether or not it needs to be treated. Nearly ½ of prostate cancers diagnosed in the U.S. fall into the low or very low risk category and have little likelihood of causing death. However, it is well known that a significant fraction of low risk cases are misclassified and actually have occult high-risk features or are destined to progress to high-risk disease. Therefore a critical need in localized prostate cancer is the development of biomarkers that predict occult or incipient aggressive disease in the low-risk population.

To address this challenge, we formed the multi-institutional Canary Tissue Microarray Project. We have used rigorous clinical trial case/cohort design, taking care to correct for institutional and spectrum biases. Funding from the Department of Defense allowed us to complete construction of the TMAs as well as the necessary infrastructure and begin testing biomarker candidates. With this infrastructure in place, we have a robust validation platform for testing prostate cancer biomarkers. Based on our success, this resource will be a source for future biomarker validation studies even after the DOD funding has ceased.

The DOD has catalyzed the formation of the infrastructure to support this project and we have now published 12 peer reviewed papers and reported our findings for nine biomarkers, **six published/in press in 2016.** Eleven additional biomarkers are under evaluations. In order to provide continuing statistical support for the ongoing activities, I have been using resource vey judiciously. I have requested another six months of no cost extension to analyze the upcoming data and help authors to address comments from journal referees. My team is actively working on data analyses and communicating back and forth with lab and clinical collaborators and we expect over the next year will complete several projects and should lead to several additional publications.

Specific Aim 1) To test markers of prognosis on prostate cancer tissue microarrays with associated clinical data.

1.A. Develop work-flow for TMA sharing, image scanning, TMA staining data analysis.

The multi-institutional TMAs have been constructed at all sites. The final TMA cohort is 1326 patients with only 31 patients excluded due to data error. We are in the process of updating follow-up on the TMAs since several years of additional follow-up have been accumulated since the cases were first selected. Patients have been selected at random from the pool of patients who had undergone radical prostatectomy at each of the sites, with special attention to selecting patients with features typical of low-intermediate risk patients seen in contemporary urologic practices. Details of patient selection, statistical considerations, and TMA construction are summarized in our publication in Advances in Anatomic Pathology published earlier this year and appended to last year's report. In addition to this cohort, a separate TMA has been constructed from 220 patients who underwent radical prostatectomy at a sister site who have very long term follow-up (up to 25 years) and hard endpoints including metastases and prostate cancer specific death. Since many of these patients were diagnosed in the pre-and early PSA eras, they are held separately as a validation cohort.

We have completed several stated aims in the proposal with regard to development of work-flow for array sharing, analysis and archiving while some aspects continue to be developed:

1) The Data Transfer Agreement (DTA) was completed between FHCRC and MDACC so the study data could be freely shared and communicated between FHCRC and MDACC. MDACC has established new database to warehouse the study data, receiving and archiving assay data from different labs/groups submitted to this project.

2) We have concluded that TACOMA algorithm as it currently stands, it is inadequate for automatic imaging reading. The main reason is that it still requires pathologists to sketch the boundary for cancer cell region. Though Dr. Tim Randolph will continue collaborating with Dr. Richard Levenson to add that functionality by another new software, it wouldn't be available in the life length of this project period to reduce pathologist reading time.

3) Data management and data analysis: We have performed data analyses for all biomarkers whose data has been submitted to MDACC. The details of the findings are summarized below.

1.B. Test candidate biomarkers of prognosis for prediction of recurrence after radical prostatectomy

In our ongoing monthly conference calls, the TMA investigators review progress and review applications for utilizing the TMAP resource. Most applications for use of the TMAs come from within the group, although it is available to the prostate cancer research community broadly and can be accessed by application through the Canary Foundation website (http://www.canaryfoundation.org). We have focused on biomarkers that have well characterized, highly performing reagents (e.g. immunohistochemical grade antibodies) and sufficient preliminary data that they could supply prognostic information independent of grade, stage and PSA. We have now completed staining for many of the biomarkers listed in our proposal and are expanding to novel biomarkers discovered since our application.

The primary objective is to correlate these two biomarkers with survival endpoints. Three survival endpoints were of interest: recurrence-free survival (RFS, where event was defined as any recurrence or metastasis or prostate cancer death), disease-specific survival (DSS, where event was defined as metastasis or prostate cancer death), and overall survival (OS, where event was defined as death of any cause).

We will first give an overall summary of the proposed assays and their status, followed by details of the findings not reported in previous progress report.

Summary of Proposed Assays and their Status

Applicant	Proposed Assay	Status
Squire/Troyer	PTEN FISH	Published
McKenney/Brooks	ERG	Published
McKenney/Brooks	SPINK1	Published
Lotan	PTEN-IF and PTEN-IHC	Both published
Tretiakova	Ki67	Published
McKenney	Histology patterns	Published
Brooks	AZGP1 in situ	Published
Brooks	AZGP1 antibody	Submitted
Brooks	MUC1	In press
Ayalo/McKenney	stromal quantification (H&E)	manuscript in prep
Brooks	ARG2 and CD38	analysis underway
Chatterjee	SULT2B	analysis of subset complete; additional proposal anticipated
Drake	N-glycan via MALDI	analysis of subset complete; additional proposal received
McKenney	Masson's trichrome	scoring underway
True	CD10	scoring underway
McKenney	p63	scoring underway
Brooks/Vakar-Lopez	p27	manuscript in prep
Brooks/Beck	HE4: prognostic model	on hold
Meng	MCM2	reviewed; conditional approval
Rohit Mehra	Schlap1	reviewed; conditional approval
Drake	N-glycan via MALDI and other	reviewed; not approved but revision requested
Liu	SMAD7	canceled; slides returned

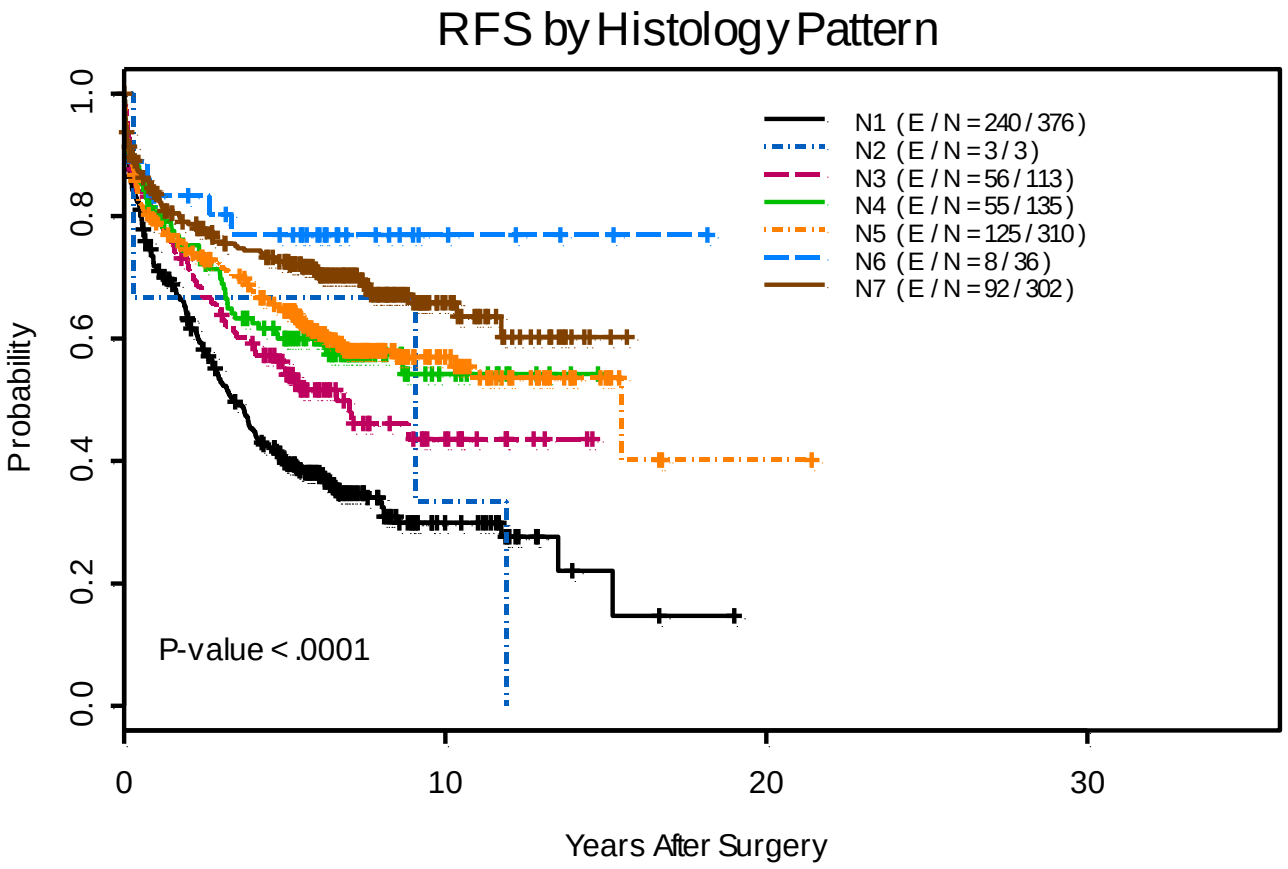
Updates on completed biomarkers not reported in previous progress reports:

1. Histological pattern is strongly associated with recurrence-free survival (RFS). Manuscript in preparation.

Table 1. Summary of multivariate Cox proportional hazard model for recurrence-free survival (RFS), where an event is defined as any recurrence, metastasis, or prostate cancer death after surgery. The first model used individual histological patterns. The second model used groups of histological patterns. Hazard ratio higher than 1 means better prognosis. Conclusions:

1. In model 1, the presence of Aw and By were significantly associated with better RFS, whereas the presence of S3 was significantly associated with worse RFS. Their effects were adjusted for margin, SVI, ECE, clinical reported Gleason score, and pre-op PSA.
2. In model 2, the presence of Aw or By was significantly associated with better RFS, whereas the presence of Ay1 or Ay2 was significantly associated with worse RFS.
3. Positive margin, SVI, ECE, Gleason higher than 3+4, and higher baseline PSA were significantly associated with worse RFS.
4. The interaction between histological patterns or groups of patterns and clinical Gleason score were not significant (results not shown), meaning that the effect of histological patterns on RFS was the same across all Gleason score groups.

Endpoint	Factor	Comparison	Hazard Ratio	95% LCL	95% UCL	P-value
RFS (N = 939 E = 429)	**HistAw**	Present vs. Absent	0.69	0.56	0.86	0.001
	HistBy	Present vs. Absent	0.78	0.62	0.98	0.03
	HistS3	Present vs. Absent	1.56	1.08	2.24	0.02
	Margin	Positive vs. Negative	1.65	1.34	2.02	<.0001
	SVI	Yes vs. No	2.03	1.48	2.78	<.0001
	ECE	Yes vs. No	1.27	1.03	1.56	0.03
	Gleason (clinical)	3+4 vs. 6	1.23	0.97	1.55	0.08
		4+3 vs. 6	1.85	1.39	2.46	<.0001
		8-10 vs. 6	1.44	1.05	1.99	0.02
	log(pre-op PSA)	1 unit increase	1.41	1.21	1.65	<.0001
	Aw/By	Either present vs. Both absent	0.66	0.54	0.81	<.0001
	Ay1/Ay2	Either present vs. Both absent	1.52	1.08	2.15	0.02
	Margin	Positive vs. Negative	1.65	1.35	2.03	<.0001
	SVI	Yes vs. No	2.00	1.46	2.74	<.0001
	ECE	Yes vs. No	1.28	1.04	1.58	0.02
	Gleason (clinical)	3+4 vs. 6	1.23	0.98	1.55	0.08
		4+3 vs. 6	1.88	1.41	2.50	<.0001
		8-10 vs. 6	1.44	1.05	1.98	0.03
	log(pre-op PSA)	1 unit increase	1.41	1.21	1.64	<.0001



Year:	0	2.5	5	7.5	10	12.5
N. Risk. N1:	376	206	131	48	20	7
N. Risk. N2:	3	2	2	2	1	0
N. Risk. N3:	113	72	53	21	13	4
N. Risk. N4:	135	92	68	26	12	4
N. Risk. N5:	310	214	180	77	38	21
N. Risk. N6:	36	27	22	10	5	3
N. Risk. N7:	302	216	192	87	33	14

N1: Ex, Ey, Ez, Dy, Dz, Cy, Cz, Bz, Ay1, Ay2 any present
N2: Not N1 and Ew present
N3: Not N1-N2 and Dx present
N4: Not N1-N3 and Bx or Cx present
N5: Not N1-N4 and Az present
N6: Not N1-N5 and Bw or Cw or Dw present
N7: Not N1-N6

2. Positive MUC1 was significantly associated with worse OS after adjusting for Gleason and age. Manuscript in preparation.

Table 2. Summary of multivariate Cox proportional hazard model for recurrence-free survival (RFS, where an event is defined as any recurrence, metastasis, or prostate cancer death), overall survival (OS, where an event is defined as death due to any cause), and disease-specific survival (DSS, where an event is defined as metastasis or prostate cancer death) for Mucin1. Hazard ratio higher than 1 means worse prognosis. Pairwise p-values were provided only if the overall p-value was significant. Conclusions:

1. **Positive MUC1 was significantly associated with worse OS after being adjusted for Gleason and age.**

Endpoint	Model	Factor	Comparison	Hazard Ratio	95% LCL	95% UCL	Pairwise P-value	Overall P-value
RFS	1	**MUC1**	Positive vs. Negative	1.14	0.92	1.42	0.23	
		Margin	Positive vs. Negative	1.64	1.32	2.03	<.0001	
		SVI	Positive vs. Negative	2.10	1.52	2.90	<.0001	
		ECE	Positive vs. Negative	1.30	1.04	1.62	0.02	
		Gleason	3+4 vs. 6	1.20	0.94	1.53	0.14	0.0001
			4+3 vs. 6	1.92	1.43	2.58	<.0001	
			8-10 vs. 6	1.50	1.07	2.09	0.02	
		Log(pre-op PSA)	1 unit increase	1.42	1.22	1.67	<.0001	
	2	**MUC1**	Strong vs. Negative	1.39	0.96	2.01	N/A	0.36
			Moderate vs. Negative	1.03	0.72	1.46	N/A	
			Weak vs. Negative	1.11	0.82	1.50	N/A	
		Margin	Positive vs. Negative	1.65	1.33	2.04	<.0001	
		SVI	Positive vs. Negative	2.04	1.47	2.83	<.0001	
		ECE	Positive vs. Negative	1.30	1.04	1.62	0.02	
		Gleason	3+4 vs. 6	1.20	0.94	1.52	0.15	0.0002
			4+3 vs. 6	1.91	1.42	2.57	<.0001	
			8-10 vs. 6	1.48	1.06	2.06	0.02	
		Log(pre-op PSA)	1 unit increase	1.42	1.22	1.67	<.0001	
OS	3	**MUC1**	Positive vs. Negative	1.82	1.06	3.11	0.03	
		Gleason	3+4 vs. 6	0.89	0.45	1.77	0.74	0.0005
			4+3 vs. 6	1.17	0.47	2.95	0.73	
			8-10 vs. 6	3.46	1.76	6.78	0.0003	
		Age	1 year increase	1.07	1.02	1.11	0.003	
	4	**MUC1**	Strong vs. Negative	1.57	0.65	3.81	N/A	0.11
			Moderate vs. Negative	2.36	1.16	4.83	N/A	
			Weak vs. Negative	1.52	0.67	3.44	N/A	
		Gleason	3+4 vs. 6	0.89	0.45	1.78	0.75	0.0004
			4+3 vs. 6	1.16	0.46	2.92	0.75	
			8-10 vs. 6	3.59	1.82	7.09	0.0002	
		Age	1 year increase	1.07	1.02	1.11	0.003	
DSS	5	**MUC1**	Positive vs. Negative	0.81	0.42	1.56	0.53	

		Gleason	3+4 vs. 6	2.70	1.18	6.18	0.02	0.001
			4+3 vs. 6	3.68	1.39	9.78	0.01	
			8-10 vs. 6	6.54	2.59	16.49	<.0001	
		Log(pre-op PSA)	1 unit increase	1.89	1.31	2.75	0.0008	
	6	**MUC1**	Strong vs. Negative	1.08	0.38	3.07	N/A	0.86
			Moderate vs. Negative	0.70	0.22	2.29	N/A	
			Weak vs. Negative	0.73	0.28	1.88	N/A	
		Gleason	3+4 vs. 6	2.68	1.17	6.13	0.02	0.001
			4+3 vs. 6	3.66	1.38	9.73	0.01	
			8-10 vs. 6	6.36	2.51	16.13	<.0001	
		Log(pre-op PSA)	1 unit increase	1.91	1.31	2.77	0.0007	
RFS	7	**MUC1**	Strong vs. Others	1.36	0.95	1.96	0.10	
		Margin	Positive vs. Negative	1.64	1.33	2.04	<.0001	
		SVI	Positive vs. Negative	2.03	1.47	2.82	<.0001	
		ECE	Positive vs. Negative	1.30	1.04	1.62	0.02	
		Gleason	3+4 vs. 6	1.20	0.94	1.53	0.14	0.0002
			4+3 vs. 6	1.92	1.43	2.58	<.0001	
			8-10 vs. 6	1.49	1.06	2.07	0.02	
		Log(pre-op PSA)	1 unit increase	1.42	1.21	1.67	<.0001	

3. Lower stromal index number and higher stroma index percent were significantly associated with worse TTBCR after adjusting for margin, SVI, ECE, and pre-op PSA. (Manuscript in preparation)

Table 3. Summary of <u>multivariate</u> Cox proportional hazard model for time-to-biochemical recurrence (<u>TTBCR)</u> for <u>stromal</u> biomarkers. Event is defined as post-op PSA above 0.2. Hazard ratio higher than 1 means worse prognosis. <u>Conclusions</u>:

1. Lower stroma index number and higher stroma index percent were significantly associated with worse TTBCR after adjusting for margin, SVI, ECE, and pre-op PSA.
2. Positive margin, SVI, ECE, and higher pre-op PSA were significantly associated with worse TTBCR.

Factor	Comparison	Hazard Ratio	95% LCL	95% UCL	P-value
Margin	pos vs. neg	1.49	1.18	1.89	0.0009
SVI	pos vs. neg	1.98	1.39	2.83	0.0002
ECE	pos vs. neg	1.52	1.20	1.93	0.001
stroma Index Num	<468.9 vs. >=468.9	1.53	1.18	1.99	0.001
stroma Index Pct	>=0.26 vs. <0.26	1.43	1.14	1.79	0.002
log(pre-op PSA)	1 unit increase	1.57	1.32	1.87	<.0001

4. Negative or weak AZGP1 IHC staining was significantly associated with worse RFS after adjusting for pre-surgery PSA, margin status, SVI, ECE, and Gleason score. Negative or weak AZGP1 CISH staining was significantly associated with worse RFS after adjusting for pre-surgery PSA, margin status, SVI, and Gleason score. (Manuscript submitted)

Table 4. Multivariate Cox proportional hazard model for recurrence-free survival (RFS) for AZGP1. RFS event is defined as any recurrence, metastasis, or prostate cancer death. Hazard ratio higher than 1 means worse prognosis. Conclusions:

1. **Negative or weak AZGP1 IHC staining was significantly associated with worse RFS after adjusting for pre-surgery PSA, margin status, SVI, ECE, and Gleason score.**
2. **Negative or weak AZGP1 CISH staining was significantly associated with worse RFS after adjusting for pre-surgery PSA, margin status, SVI, and Gleason score.**

Model	Factor	Comparison	Hazard Ratio	95% LCL	95% UCL	P-value
1 (Total #Pts = 835, #Events = 382	**AZGP1 IHC**	Negative/Weak vs. Moderate/Strong	1.39	1.13	1.71	0.002
	Log(PSA)	1 unit increase	1.43	1.21	1.68	<.0001
	margin	Pos vs. Neg	1.62	1.31	2.02	<.0001
	SVI	Pos vs. Neg	2.20	1.58	3.06	<.0001
	ECE	Pos vs. Neg	1.26	1.01	1.58	0.04
	Gleason	3+4 vs. <=6	1.19	0.93	1.52	0.16
		4+3 vs. <=6	1.99	1.47	2.69	<.0001
		8-10 vs. <=6	1.43	1.02	1.99	0.04
2 (Total #Pts = 811, #Events = 377)	**AZGP1 CISH**	Negative/Weak vs. Moderate/Strong	1.28	1.04	1.58	0.02
	Log(PSA)	1 unit increase	1.46	1.24	1.73	<.0001
	margin	Pos vs. Neg	1.71	1.39	2.12	<.0001
	SVI	Pos vs. Neg	2.26	1.62	3.15	<.0001
	Gleason	3+4 vs. <=6	1.22	0.96	1.57	0.11
		4+3 vs. <=6	2.12	1.57	2.86	<.0001
		8-10 vs. <=6	1.60	1.15	2.23	0.006

5. Ki67 status is significantly associated with OS and DSS (Manuscript submitted)

Table 5. Summary of multivariate Cox proportional hazard model for recurrence-free survival (RFS), overall survival (OS), and disease-specific survival (DSS) by Ki-67 status.						
Model	Factor	Comparison	Hazard Ratio	95% LCL	95% UCL	Overall p-value
RFS Model 1 (N = 634, #Events = 281)	**Ki67% weighted average**	1% increase	1.07	1.03	1.11	0.0008
	Margin	Pos vs. Neg	1.41	1.09	1.84	0.01
	Seminal vesicle invasion	Yes vs. No	1.85	1.23	2.80	0.003
	Gleason score	3+4 vs. 6	1.22	0.90	1.66	0.005
		4+3 vs. 6	1.88	1.33	2.66	
		8-10 vs. 6	1.42	0.96	2.09	
	Pathologic stage	pT3/pT4 vs. pT2	1.43	1.07	1.92	0.02
	Log(PSA)	1 unit increase	1.62	1.35	1.96	<.0001
RFS Model 2 (N = 634, #Events = 281)	**Ki67% maximum**	1% increase	1.04	1.01	1.07	0.007
	Margin	Pos vs. Neg	1.40	1.08	1.83	0.01
	Seminal vesicle invasion	Yes vs. No	1.85	1.22	2.79	0.004
	Gleason score	3+4 vs. 6	1.24	0.91	1.67	0.003
		4+3 vs. 6	1.91	1.35	2.70	
		8-10 vs. 6	1.46	0.99	2.15	
	Pathologic stage	pT3/pT4 vs. pT2	1.44	1.07	1.93	0.02
	Log(PSA)	1 unit increase	1.61	1.34	1.95	<.0001
OS (N = 984, #Events = 57)	**Ki67% positive**	1% increase	1.09	1.01	1.16	0.02
	Gleason Score	3+4 vs. <=6	0.87	0.44	1.72	0.68
		4+3 vs. <=6	1.14	0.46	2.84	0.78
		8-10 vs. <=6	3.28	1.65	6.51	0.0007
DSS (N = 874, #Events = 44)	**Ki67% positive**	1% increase	1.10	1.02	1.18	0.02
	Log(PSA)	1 unit increase	1.98	1.35	2.89	0.005
	Gleason Score	3+4 vs. <=6	2.27	0.93	5.52	0.07
		4+3 vs. <=6	2.75	0.97	7.81	0.06
		8-10 vs. <=6	5.13	1.92	13.75	0.001

6. P27 is not significantly associated with RFS after adjusting for clinical predictors (Manuscript in preparation)

Table 6. Summary of multivariate Cox proportional hazard model results for RFS by p27 status. RFS event is defined as any recurrence, metastasis, or prostate cancer death post-surgery. Hazard ratio higher than 1 means worse prognosis. The total sample size for both models was 699, and the number of RFS events observed was 319. Conclusion:

1. **p27 was not significantly associated with RFS after adjusting for margin, SVI, Gleason, and pre-op PSA.**

Factor	Comparison	Hazard Ratio	95% LCL	95% UCL	P-value
p27HscoreCyto	**1 unit increase**	0.999	0.997	1.000	0.16
Margin	**pos vs. neg**	1.61	1.28	2.03	<.0001
SVI	**yes vs. no**	2.50	1.74	3.59	<.0001
Gleason	**3+4 vs. 6**	1.44	1.09	1.90	0.01
	4+3 vs. 6	2.17	1.56	3.01	<.0001
	8-10 vs. 6	1.73	1.21	2.49	0.003
Log(pre-op PSA)	**1 unit increase**	1.57	1.32	1.86	<.0001
p27HscoreCyto	**1 unit increase**	0.999	0.997	1.000	0.11
Margin	**pos vs. neg**	1.62	1.29	2.03	<.0001
SVI	**yes vs. no**	2.50	1.74	3.60	<.0001
Gleason	**3+4 vs. 6**	1.43	1.08	1.89	0.01
	4+3 vs. 6	2.15	1.55	2.99	<.0001
	8-10 vs. 6	1.73	1.21	2.48	0.003
Log(pre-op PSA)	**1 unit increase**	1.56	1.31	1.86	<.0001

7. CD38 IHC is not significantly correlated with any survival endpoint after adjusting for factors in each model.

Table 7. Summary of multivariate Cox proportional hazard models for RFS, OS, and DSS. Hazard ratio higher than 1 means worse prognosis. Conclusion:
1. CD38 IHC was not significantly associated with any survival endpoint after adjusting for other factors.

Endpoint	Factor	Comparison	Hazard Ratio	95% LCL	95% UCL	P-value
RFS	**CD38 IHC**	**Moderate/Strong vs. Weak/Negative**	0.827	0.671	1.018	0.07
	Margin	**Pos. vs. Neg.**	1.685	1.353	2.099	<.0001
	SVI	**Pos. vs. Neg.**	2.256	1.623	3.138	<.0001
	ECE	**Pos. vs. Neg.**	1.287	1.028	1.612	0.03
	Gleason	**3+4 vs. 6**	1.207	0.941	1.549	0.14
		4+3 vs. 6	1.919	1.407	2.617	<.0001
		8-10 vs. 6	1.522	1.094	2.118	0.01
	Log(pre-op PSA)	**1 unit increase**	1.437	1.219	1.695	<.0001
OS	**CD38 IHC**	**Moderate/Strong vs. Weak/Negative**	0.787	0.454	1.366	0.39
	Gleason	**3+4 vs. 6**	0.78	0.38	1.602	0.50
		4+3 vs. 6	1.362	0.542	3.421	0.51
		8-10 vs. 6	3.105	1.566	6.155	0.001
	Age	**1 year increase**	1.059	1.015	1.104	0.01
DSS	**CD38 IHC**	**Moderate/Strong vs. Weak/Negative**	0.941	0.516	1.715	0.84
	Gleason	**3+4 vs. 6**	2.296	0.99	5.323	0.053
		4+3 vs. 6	2.865	1.021	8.035	0.046
		8-10 vs. 6	5.476	2.175	13.788	0.0003
	Log(pre-op PSA)	**1 unit increase**	1.985	1.351	2.917	0.0005

8. CD10 is significantly associated with overall survival after adjusting for Gleason and age.

Table 8. Summary of <u>multivariate</u> Cox proportional hazard model results by endpoint. Hazard ratio higher tha prognosis. LCL = lower confidence limit, UCL = upper confidence limit. <u>Conclusions</u>:

1. **Positive CD10 was significantly associated with better OS after adjusting for Gleason and age. CD10 w associated with RFS or DSS after adjusting for margin, SVI, ECE, Gleason, and pre-op PSA.**
2. **Positive margin, SVI, ECE, Gleason higher than 4+3, and higher pre-op PSA were significantly associate**
3. **Gleason higher than 4+3 and higher pre-op PSA were significantly associated with worse DSS.**
4. **Gleason higher than 8 and older age were significantly associated with worse OS.**

Endpoint	Factor	Comparison	Hazard Ratio	95% LCL	95%
RFS	CD10 Any Positive	Yes vs. No	1.198	0.966	
	Margin	Yes vs. No	1.667	1.333	
	SVI	Yes vs. No	2.031	1.447	
	ECE	Yes vs. No	1.386	1.101	
	Gleason score	3+4 vs. <=6	1.163	0.9	
		4+3 vs. <=6	1.759	1.283	
		8-10 vs. <=6	1.388	0.983	
	Log(Pre-op PSA)	1 unit increase	1.409	1.19	
OS	CD10 Any Positive	Yes vs. No	0.462	0.25	
	Gleason score	3+4 vs. <=6	0.802	0.369	
		4+3 vs. <=6	1.768	0.684	
		8-10 vs. <=6	3.73	1.82	
	Age	1 year increase	1.059	1.013	
DSS	CD10 Any Positive	Yes vs. No	1.338	0.713	
	Gleason score	3+4 vs. <=6	2.157	0.923	
		4+3 vs. <=6	2.876	1.057	
		8-10 vs. <=6	5.317	2.105	
	Log(Pre-op PSA)	1 unit increase	1.964	1.34	

9. Multi-biomarker panel identification. We used all biomarkers analyzed so far (PTEN, Ki-67, ERG, SPINK, AZGP1, stroma index, histology pattern, CD38, ARG2, CD10, and p27) and available patient characteristics (age, pre-op PSA, Gleason, margin, SVI, and ECE) to perform backwards elimination and identified two final models for RFS.

Table 9a. Summary of multivariate Cox proportional hazard model for RFS. We used backwards elimination procedure started with all biomarkers and continued until only significant factors remained in the model. Hazard ratio higher than 1 means worse prognosis. Effect of N6 could not be estimated in this multivariate model because no RFS event was observed in the modeled subset (N = 635, E = 287). Conclusions:

1. Positive margin, SVI, high Ki-67 %pos, negative SPINK, negative/weak AZGP1 (IHC), histology groups N1-N5, and higher pre-op PSA were significantly associated with worse RFS after adjusting for each other's effects.

Factor	Comparison	Hazard Ratio	95% LCL	95% UCL	P-value
Margin	Pos vs. Neg	1.679	1.318	2.139	<.0001
SVI	Yes vs. No	2.292	1.563	3.361	<.0001
Ki-67 Weighted Avg %Pos	>=5% vs. <5%	1.498	1.121	2.001	0.0063
SPINK	Neg vs. Pos	5.026	1.589	15.896	0.006
AZGP1(IHC)	Negative/Weak vs. Moderate/Strong	1.331	1.002	1.766	0.048
Histology Group	N1 vs. N7	3.03	1.865	4.924	<.0001
	N2 vs. N7	5.895	1.708	20.344	0.005
	N3 vs. N7	2.027	1.144	3.593	0.0155
	N4 vs. N7	2.51	1.456	4.328	0.0009
	N5 vs. N7	1.774	1.07	2.94	0.0263
	N6 vs. N7	N/A	N/A	N/A	N/A
Log(Pre-op PSA)	1 unit increase	1.568	1.311	1.876	<.0001

Table 9b. Summary of another multivariate Cox proportional hazard model for RFS. We used backwards elimination procedure started with all biomarkers except for Ki-67 (labor intensive assay) and PTEN FISH (more than 50% unknown), and continued until only significant factors remained in the model. Hazard ratio higher than 1 means worse prognosis. Sample size (N = 796, E = 368) in this model is different from the one in table 9a. Conclusions:

1. Positive margin, SVI, any PTEN loss by IHC, negative/weak AZGP1 (IHC), histology groups N1-N5, and higher pre-op PSA were significantly associated with worse RFS after adjusting for each other's effects.

Factor	Comparison	Hazard Ratio	95% LCL	95% UCL	P-value
Margin	Pos vs. Neg	1.884	1.521	2.334	<.0001
SVI	Yes vs. No	2.088	1.495	2.915	<.0001
PTEN(IHC)	Any PTEN Loss vs. Intact	1.282	1.02	1.611	0.0334
AZGP1(IHC)	Negative/Weak vs. Moderate/Strong	1.316	1.023	1.694	0.0326
Histology Group	N1 vs. N7	2.558	1.696	3.857	<.0001
	N2 vs. N7	3.651	1.104	12.076	0.0339
	N3 vs. N7	1.875	1.159	3.034	0.0104
	N4 vs. N7	2.043	1.273	3.281	0.0031
	N5 vs. N7	1.629	1.065	2.49	0.0243
	N6 vs. N7	0.918	0.353	2.387	0.8606
Log(Pre-op PSA)	1 unit increase	1.464	1.239	1.728	<.0001

Ongoing studies: See summary on page 3-4. Preliminary analyses completed for ARG2 and CD38, SULT2B, N-glycan. Continuing statistical support for manuscripts reporting MUC1, stromal index, histology pattern, and p27, and second manuscript on PTEN. Expecting statistical analyses once the pathologist scoring completed for CD10, p63, Masson's trichrome.

Specific Aim 2) To evaluate candidate markers that correlate with Gleason grade on prostate cancer tissue microarrays with associated clinical data.

Thus far, we have focused on building the analysis pipeline and in staining high priority biomarkers of prognosis. In all of the biomarkers we have tested thus far, we have interrogated each for its correlation with Gleason score. In general, most of them are correlated, although not completely. While these do not address the intent of this Aim, we are not disappointed since it does appear that *these biomarkers are supplying prognostic information that is independent of Gleason score.* The intent of Aim 2, on the other hand, was to investigate biomarkers that correlate with Gleason grade. Several

markers are in our queue and are listed in the original proposal. For some, we are still looking for high quality affinity reagents that provide interpretable staining with limited background. Leading candidates are AGR2, a marker expressed at high levels in Gleason pattern 3 cancers and Monoamine oxidase A, expressed at high levels in Gleason pattern 4 disease. As we get through our candidate prognostic markers (listed above and in the queue) we will refocus on biomarkers that predict Gleason grade. This could be useful in characterizing biopsy samples to predict upgrading.

However, this clinical question might become less relevant in the future since several tools have been developed that already predict up-grading. For example the OncotypeDx assay has been calibrated and already validated precisely for this purpose. In addition, multiparametric MRI shows good correlation with grade in that only the high-grade lesions are visible, while the low grade lesions are not. As the clinical practice evolves, we will decide whether we wish to continue to pursue development of IHC biomarkers that predict Gleason score

For all biomarkers, whether for Gleason score or prognosis, the statistical analysis strategy has been outlined in our proposal and will be used as soon as reads are available from the pathologists, both in their correlations with Gleason score and in their complementary property with Gleason score.

Key Research Accomplishments

- Provided statistical expertise in biomarker review and approval by the investigative team to ensure quality of the reagents and sufficient level of evidence for investigation of a particular biomarker on our valuable resource.
- Data receiving, reconcile data questions, and archiving at MDACC.
- Received final clinical data that will be used for analysis of biomarker performance to the MD Anderson DMCC.
- Established and tested the data analysis pipeline for anticipated additional biomarker data.
- Evaluated TACOMA imaging analysis algorithm using Survivin, CD117, and ERG data and concluded that it is inadequate for automated imaging analysis as it stands along.
- Completion of analysis of PTEN FISH and a manuscript published.
- Completion of analysis of Ki67 PI and a manuscript published.
- Completion of analysis of ERG IHC and PTEN IHC and presentation at international meetings and results published.
- Completion of analysis of SPINK and results published.
- Completion of analysis of AZGP1 results published.
- Completion of analysis of a modified Gleason grading system with Jesse McKenney, manuscript published.
- Completion of analysis of Muc1 and results published.
- Ongoing analysis of stromal quantification, ARG2, CD38, SULT2B, N-glycan via MALDI, Masson's trichrome, CD10, p63, and p27. We expect all of these, regardless of outcome (prognostic or not) will be submitted as separate publications.
- Significant preliminary data from this collaboration that will position us well for the next phase of funding.

Reportable Outcomes

1) Publications referencing this grant:

James D. Brooks: Translational genomics: The challenge of developing cancer diagnostic biomarkers. *Genome Research* **22:** 183-187, 2012.

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J James D. Brooks: Managing localized prostate cancer in the era of prostate specific antigen testing. *Cancer* **119:** 3906-3909, 2013.

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Troyer D, Jamaspishvili T, Wei W, Feng Z, Good J, Hawley S, Fazli L, McKenney J, Simko J, Hurtado-Coll A, Carroll P, Gleave M, Lance R, Lin D, Nelson P, Thompson I, True L, Brooks J, Squire J. A multicenter study shos PTEN deletion is strongly associated with seminal vesicle involvement and extracapsular extension in localized prostate cancer. The Prostate. 75(11): 1206-1215, 2015.

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MS Tretiakova, W Wei, HD Boyer, LF Newcomb, S Hawley, H Auman, F Vakar-Lopez, JK McKenney, L Fazli, J Simko, DA Troyer, A Hurtado-Coll, IM Thompson Jr., PR Carroll, WJ Ellis, ME Gleave, PS Nelson, DW Lin, LD True, Z Feng, JD Brooks, Prognostic value of Ki67 in

localized prostate carcinoma: a multi-institutional study of >1,000 prostatectomies, **2016**, *Prostate Cancer and Prostatic Diseases*, 19(3): 264-270

TL Lotan, W Wei, O Ludkovski, CL Morais, LB Guedes, T Jamaspishvili, K Lopez, ST Hawley, Z Feng, L Fazli, A Hurtado-Coll, JK McKenney, J Simko, PR Carroll, M Gleave, DW Lin, PS Nelson, IM Thompson, LD True, JD Brooks, R Lance, DA Troyer, JS Squire, Analytic validation of a clinical-grade PTEN immunohistochemistry assay in prostate cancer by comparison with PTEN FISH, **2016**, *Modern Pathology*, 29(8): 904-914

JD Brooks, W Wei, JR Pollack, RB West, J Sunwoo, JH Shin, SJ Hawley, H Auman, LF Newcomb, J Simko, A Hurtado-Coll, DA Troyer, PR Carroll, ME Gleave, DW Lin, PS Nelson, IM Thompson, LD True, JK McKenney, Z Feng, L Fazli, Loss of expression of AZGP1 is associated with worse clinical outcomes in a multi-institutional radical prostatectomy cohort, **2016**, *Prostate*, 76(15): 1409-1419

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Conclusion

We have undertaken a challenging task of creating a multi-institutional TMA resource with rigorous case/cohort design. To our knowledge, such a resource has not been previously created and offers the advantage of reducing institutional biases as well as spectrum biases. In the uniform design and through image acquisition and archiving technologies, we have created a resource that can be easily used by the greater prostate cancer research community. In many ways, this resource represents a gold standard by for evaluation of prognostic biomarkers. We have completed all phases of pipeline construction and continue to refine our work-flow to improve functionality as we work with the resource. We now have tested several biomarkers and confirmed that they are prognostic, and resulted 9 peer reviewed publications. In addition, we will continue to carry out analysis of new biomarkers and solicit applications for biomarkers inside and outside our research group. This research directly addresses the PCRP overarching challenge to *distinguish lethal from indolent disease*.