Award Number: W81XWH-11-1-0489

TITLE: PREDICTING PROSTATE CANCER PROGRESSION AT TIME OF DIAGNOSIS

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REPORT DATE: June 2015

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
June 2015	ANNUAL REPORT	15Jun2014 – 14Jun2015

4. TITLE AND SUBTITLE
"PREDICTING PROSTATE CANCER PROGRESSION AT TIME OF DIAGNOSIS"

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0489

5c. PROGRAM ELEMENT NUMBER
–

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5d. PROJECT NUMBER
–

5e. TASK NUMBER
–

5f. WORK UNIT NUMBER
–

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER
–

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
–

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
–

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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 in the next year.

15. SUBJECT TERMS
Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	14	19b. TELEPHONE NUMBER *(include area code)*

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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 have completed accession and processing of all specimens from both UCSF and UW. As noted last year, 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.

We have completed the pathology work required for tissue identification, re-reading, and punching cases on N=291 cases from UCSF and N=192 cases from UW. N=118 from UCSF and N=82 from UW were upgraded cases and included samples of both Gleason pattern 3 and Gleason pattern 4. Thus the total sample N=674.

All cases have been cut and sent to the Paris lab and to Myriad genetics for DNA and RNA extraction, respectively. Nucleic acid yields and quality control checks continue to appear excellent.

Task 2: Blood, urine, and tissue 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. The specified analyses in this grant will focus on the first 450 enrollees. Over 165 of these 450 have progressed by study criteria, which is consistent with baseline expectations when the statistical plan was generated. 143 men (87% of those reclassified) reclassified due to Gleason grade increase on subsequent biopsy, and 22 (13%) for increase in biopsy tumor volume. 87 of these men (19%) have gone on to prostatectomy and have surgical pathology results available.

Task 3: Serum analyses (Aims 1 and 2)

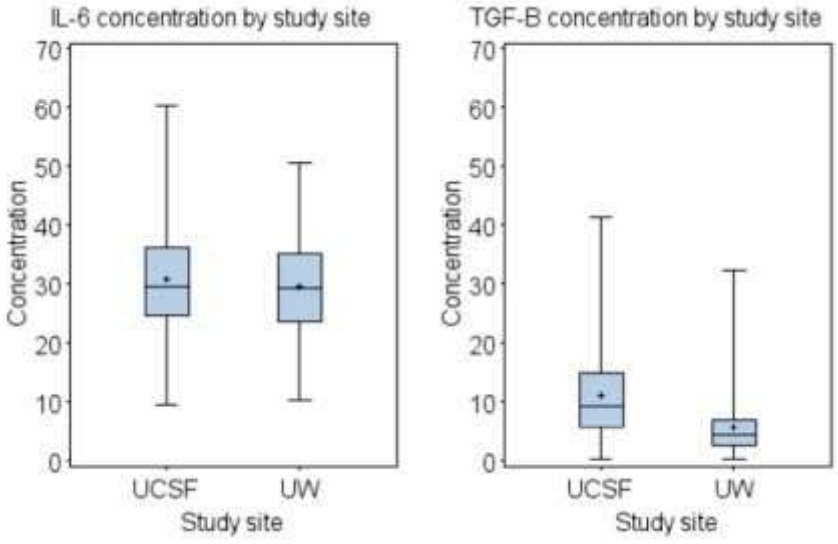
We have now completed all TGFβ1 and IL6SR analyses on the N=397 UCSF Aim 1 specimens, N=260 UW specimens, (Table 1) and the N=505 PASS Aim 2 specimens. All patients were diagnosed in 2000 or later with low risk disease (diagnosis PSA < 10 ng/ml, clinical stage T1-2, biopsy Gleason grade 2-6) and underwent radical prostatectomy monotherapy within 6 months. In the past year, we further undertook to repeat analyses on the UCSF specimens given an unexpected finding of different mean scores between the two cohorts and the Canary (Aim 2) specimens. The repeat analysis are more consistent with the other cohorts, and likely represent batch differences in the ELISA plates used.

Table 1. Aim 1 Cohort characteristics at diagnosis among 657 men with low-risk prostate cancer at UCSF and UW

DIAGNOSIS	UCSF N	MEAN	SD	UW N	MEAN	SD	ANOVA P-VAL
AGE	397	58.6	6.85	260	59.1	7.05	0.38
PSA	397	5.4	1.89	260	5.0	1.83	<.01
% POS CORS	396	29.4	21.37	260	26.5	17.98	0.07

Normalization of TGFβ1 levels for PF4 levels as described last year 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.

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



Primary outcome at RP were rates of upgrade (UG) to Gleason 3+4 or higher or upstage (US) to pT3/4. A prespecified subset analysis also analyzed "major" upgrading to Gleason ≥4+3 and/or ≥pT3b (Table 3).

Table 2. Rates of upgrading and upstaging at radical prostatectomy among UCSF and UW men with clinically low-risk prostate cancer

OUTCOMES	VALUE	UCSF N	(%)	UW N	(%)	CHISQ P-VAL
ANY UPGRADE	No change	226	57	139	53	0.19
	Minor increase	129	33	101	39	.
	Major increase	41	10	20	8	.
	Missing	1	.	19	.	.
MAJOR UPGRADE	No change	355	90	240	92	0.25
	Major increase	41	10	20	8	.
	Missing	1	.	19	.	.
UPSTAGE	No change	336	85	240	92	0.01
	Minor increase	49	12	17	7	.
	Major increase	12	3	3	1	.
	Missing	0	.	19	.	.
MAJOR CHANGE	No upstage or upgrade	349	88	238	92	0.35
	Upstage only	7	2	2	1	.
	Upgrade only	36	9	19	7	.
	Both upstage and upgrade	5	1	1	0	.
	Missing	0	.	19	.	.
ANY CHANGE	No upstage or upgrade	209	53	136	52	<.01
	Upstage only	17	4	3	1	.
	Upgrade only	126	32	104	40	.
	Both upstage and upgrade	44	11	17	7	.
	Missing	1	.	19	.	.
OUTCOME GROUP	None	210	53	136	52	0.26
	Minor UG/US	139	35	102	39	.
	Major UG/US	48	12	22	8	.
	Missing	0	.	19	.	.

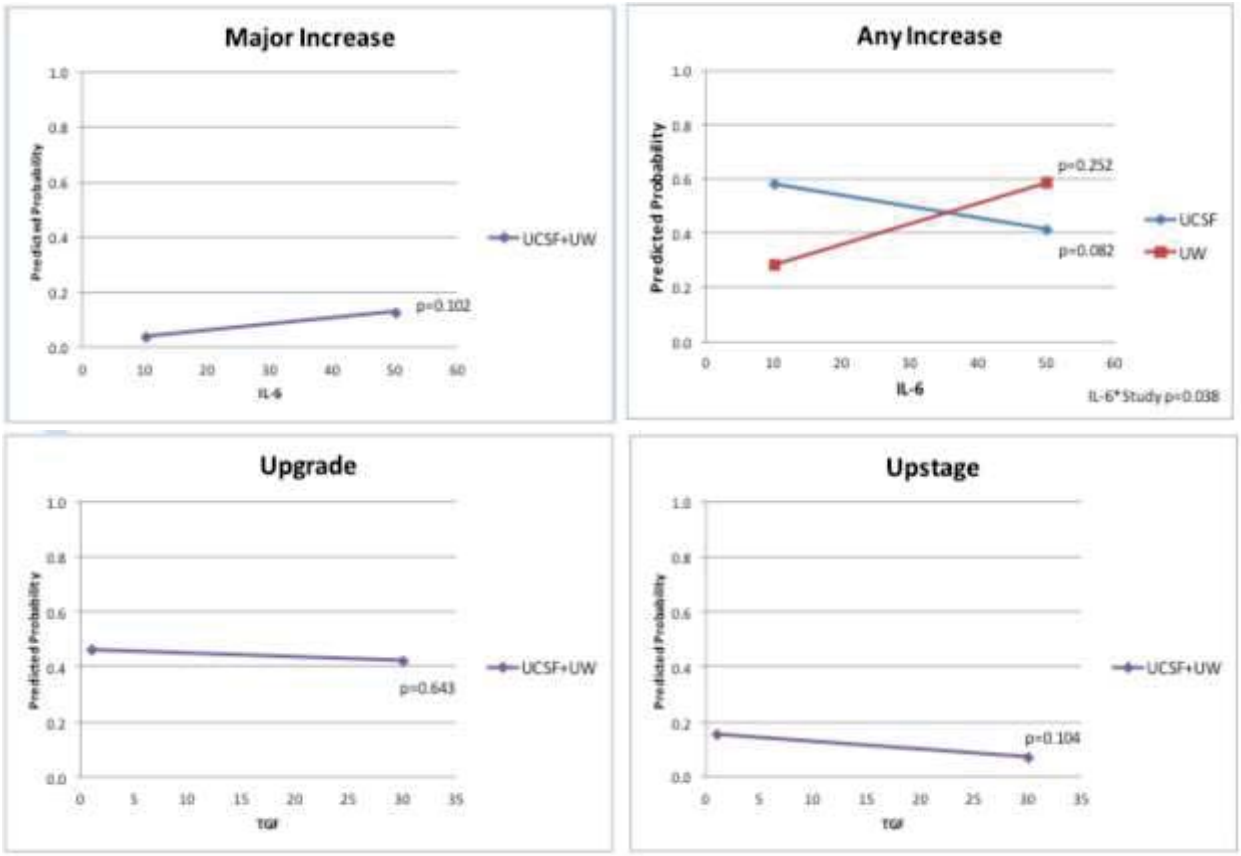
Each biomarker was analyzed in logistic regression models adjusting for age, percent biopsy cores positive, PSA, and study site. Despite re-processing the UCSF specimens as described above, we found unexpected statistical interaction between IL-6SR and study site (UCSF vs. UW). On multivariable analysis, IL-6SR associated with upgrading/upstaging in opposite directions between the two cohorts, though neither was statistically significant (Table 3, Figure 2).

Table 3: 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).



Our remaining analysis will be to repeat the analyses using multinomial regression with 3 possible outcomes (no change / minor upgrading or upstaging / major upgrading or upstaging). These will be completed in the next few weeks.

Task 4

As noted last year, N=500 PASS participants (Table 4) 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). Preliminary analyses suggest positive associations between the urinary markers and baseline tumor characteristics. A paper describing associations between these urine markers and baseline risk characteristics on the first N=387 men in PASS has been published (Lin et al, Clin Cancer Res 2013; manuscript included previously).

Table 4: Patient characteristics from the 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

Table 5: Distribution of serum IL-6SR, serum TGFb1, urine PCA3, and urine T2-ERG levels in the PASS cohort

	N	Mean	Std.	Min	Q1	Median	Q3	Max	Q. range	Range
Diagnosis to Baseline (yr)	500	1.35	1.49	0.02	0.38	0.71	1.88	8.65	1.50	8.63
IL6	500	47.95	16.68	17.89	35.52	45.58	54.99	166.55	19.47	148.65
TGF	500	3.74	3.64	0.05	1.69	2.57	4.29	30.74	2.60	30.69
logPCA3 score	500	3.43	0.95	-1.61	2.83	3.41	4.04	7.17	1.21	8.78
logT2-ERG score	500	1.60	3.86	-9.21	0.98	2.55	4.00	7.64	3.02	16.85

In the next 2 months we will obtain updated followup data on these patients to maximize the reclassification event rate, and will finalize the analyses of the markers in relation to our previously described endpoints.

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 on 531 (79%) of the specimens, and array comparative genomic hybridization (aCGH) analysis on 471 (70%) of the specimens. Work is proceeding rapidly on the remaining specimens, and we anticipate all extraction and aCGH analysis to be completed within the next 2 months. 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.

Myriad has completed RNA extraction on approximately half the specimens and anticipates completion within the next 3 months. We will receive the Prolaris scores as a batch when all are completed, and will also receive residual RNA back for future analyses.

Task 6

As noted previously, the VSIMS database has been updated to accommodate new tissue-based data fields, and biomarker data are being entered as they become available. We are awaiting maximal follow-up in the PASS cohort before finalizing any biomarker analyses in this cohort. Likewise, while we have performed preliminary serum studies among the UCSF Aim 1 specimens, we await results from the UW specimens before finalizing these or preparing publications. In the meantime, we have continued analyses of PSA data from PASS participants with the intent of better understanding of PSA kinetics in the active surveillance setting. We subsequently have received and processed 1762 full quality of life surveys, of which 550 also include detailed diet and lifestyle information. These results will be analyzed together with the biomarker data this fall in terms of their ability to predict progression to treatment.

Key Research Accomplishments

- As noted previously, 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, as they associate closely with known clinical markers.

- IL-6 and TGFβ1, markers previously associated with biochemical recurrence after prostatectomy for relatively high risk disease, do not appear to be predictive of early endpoints of upgrading/upstaging from clinically low risk disease or early reclassification on active surveillance, though we do plan additional analyses before this section of the grant is concluded.
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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 submitted 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 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 together with the markers already underway with others and studies under this award*. The revised grant, R01 CA198145-01A1, received an impact score of 22, corresponding to 7%ile. We are optimistic regarding a final funding decision on this proposal.

Conclusion

We have made additional progress during the 4th year of this project (no cost extension). All plasma and tissue specimens have been collected and distributed to the various labs. Plasma and urine processing is complete for both Aims. Tissue processing and DNA and RNA extraction and analysis is well underway and should be complete within the next three months.

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 in progress under this grant. 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 continuing support of the DOD and the CDMRP.

We look forward to completing our analyses and reporting results.

University of California
San Francisco

Human Research Protection Program
Committee on Human Research

Notification of Expedited Review Approval

Principal Investigator
Peter R Carroll

Type of Submission: Continuing Review Submission Form
Study Title: Predicting Prostate Cancer Progression at Time of Diagnosis

IRB #: 11-07012
Reference #: 138940

Committee of Record: Parnassus Panel

Study Risk Assignment: Minimal

Approval Date: 06/02/2015 **Expiration Date:** 06/26/2016

Regulatory Determinations Pertaining to this Approval:

- The requirement for individual Research HIPAA Authorization is waived for all subjects. The use or disclosure of the requested information does not adversely affect the rights and welfare of the individuals and involves no more than a minimal risk to their privacy based on, at least, the presence of the following elements: (1) an adequate plan to protect the identifiers from improper use and disclosure; (2) an adequate plan to destroy the identifiers at the earliest opportunity consistent with conduct of the research, unless there is a health or research justification for retaining the identifiers or if such retention is otherwise required by law; (3) adequate written assurances that the requested information will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other research for which the use or disclosure of the requested information would be permitted by the Privacy Rule; (4) the research could not practicably be conducted without the waiver; and (5) the research could not practicably be conducted without access to and use of the requested information.
- A waiver or alteration of informed consent is acceptable because, as detailed in the application: (1) the research involves no more than minimal risk to the subjects; (2) the waiver or alteration will not adversely affect the rights and welfare of the subjects; (3) the research could not practicably be carried out without the waiver or alteration; and (4) whenever appropriate, the subjects will be provided with additional pertinent information after participation.
- The waiver or alteration of informed consent applies to all subjects.

All changes to a study must receive CHR approval before they are implemented. Follow the modification request instructions. The only exception to the requirement for prior CHR review and approval is when the changes are necessary to eliminate apparent immediate hazards to the subject (45 CFR 46.103.b.4, 21 CFR 56.108.a). In such cases, report the actions taken by following these instructions.

Expiration Notice: The iRIS system will generate an email notification eight weeks prior to the expiration of this study's approval. However, it is your responsibility to ensure that an application for continuing review approval has been submitted by the required time. In addition, you are required to submit a study closeout report at the

completion of the project.

Approved Documents: To obtain a list of documents that were approved with this submission, follow these steps: Go to My Studies and open the study – Click on Submissions History – Go to Completed Submissions – Locate this submission and click on the Details button to view a list of submitted documents and their outcomes.

For a list of all currently approved documents, follow these steps: Go to My Studies and open the study – Click on Informed Consent to obtain a list of approved consent documents and Other Study Documents for a list of other approved documents.

San Francisco Veterans Affairs Medical Center (SFVAMC): If the SFVAMC is engaged in this research, you must secure approval of the VA Research & Development Committee in addition to CHR approval and follow all applicable VA and other federal requirements. The CHR website has more information.