American College of Radiology

Annual Progress Report: 2012 Formula Grant

Reporting Period

July 1, 2014 – June 30, 2015

Formula Grant Overview

The American College of Radiology received $1,851,408 in formula funds for the grant award period January 1, 2013 through December 31, 2016. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Exploration of the RTOG Clinical Trial Database – Beyond Protocol-Specified Endpoints – For over 40 years, the Radiation Therapy Oncology Group (RTOG) has been funded by the National Cancer Institute (NCI) to conduct clinical trials seeking to improve the survival and quality of life of cancer patients. Drawing upon this vast resource of demographic, treatment, and outcome data, the researchers will test new hypotheses and explore associations that were not defined in the treatment protocols for patients with gynecologic, head and neck, lung, and prostate cancers. These analyses may inform and/or lead to future protocols.

Anticipated Duration of Project

7/1/2013 – 12/31/2016

Project Overview

This project aims to analyze data that have been collected in previous RTOG studies. The specific research objectives of this project relate to six data analysis efforts.

Aim 1: Correlation of Radiation Therapy Dose Volume Histogram (DVH) Data with GI Toxicity in Post-Operative Cervical and Endometrial Cancer Patients Treated with IMRT: RTOG 0418 is a Phase II trial that evaluated the use of IMRT in post-operative cervical and endometrial cancer patients. Using data collected from this trial, we will correlate the radiation therapy DVH data, relative to the amount of bowel receiving radiation, with reported GI adverse events.

Aim 2: Evaluation of the Impact of Treatment Time for Head and Neck Cancer Patients Treated with Radiation Therapy: Using data collected from 3 RTOG Phase III Head and Neck Cancer Trials (RTOG 9003, 9111, and 9501), we will evaluate whether or not a longer radiotherapy treatment time is associated with a significantly worse clinical outcome.

Note: This project is funded, in part, under a grant with the Pennsylvania Department of Health. The Department specifically disclaims responsibility for any analyses, interpretations or conclusions.

Aim 3: Evaluation of Outcome in Squamous Cell Carcinoma of the Head and Neck (SCCHN) Based on Age: Using data collected from several RTOG combined modality Head and Neck cancer trials (RTOG 8527, 9003, 9111, 9703, 9903, 9914, 0129, and 0522), we will evaluate efficacy outcomes & Adverse Events (AE) by age categorizations: ≥ 70 vs. < 70 and ≤ 60 vs. 61-69 vs. ≥ 70.

Aim 4: Evaluation of Incidental Cardiac Irradiation on Toxicity and Survival in Stage IIIA/IIIB Non-Small Cell Lung Cancer (NSCLC) Patients Treated with Chemoradiotherapy: Using data from RTOG 0617, we will correlate the radiation therapy dose volume histogram data, relative to the amount of heart receiving radiation, with cardiac and pulmonary AEs and overall survival.

Aim 5: Evaluation of Hormone Therapy Length on Outcome for Intermediate Risk Prostate Cancer Patients: We will evaluate whether or not radiotherapy with long-term hormones (28 months) is associated with better outcome than radiotherapy with short-term hormones (4 months) for the RTOG 9202 subset of intermediate-risk prostate cancer patients.

Aim 6: Evaluation of Changes in Serum Testosterone Levels in Prostate Cancer Patients Treated with Radiotherapy Alone: We will evaluate associations between radiated area (prostate vs. whole pelvis) and changes in serum testosterone for the patients treated on the radiotherapy alone arm of RTOG 9408.

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Expected Research Outcomes and Benefits

Aim 1: Correlation of Radiation Therapy Dose Volume Histogram Data with GI Toxicity in Post-Operative Cervical and Endometrial Cancer Patients Treated with IMRT: Results from this aim will inform dose constraints for future IMRT GYN trials to help minimize GI adverse events.

Aim 2: Evaluation of the Impact of Treatment Time for Head and Neck Cancer Patients Treated with Radiation Therapy: Results from this aim may impact the approach to treatment breaks and will help to inform treatment time components of future trials.

Aim 3: Evaluation of Outcome in Squamous Cell Carcinoma of the Head and Neck (SCCHN) Based on Age: Results from this aim may identify subsets of patients by age that are associated with a benefit from certain treatment regimens and/or associated with significantly better/worse treatment adverse events. This will help to form the basis for future SCCHN clinical trials.

Aim 4: Evaluation of Incidental Cardiac Irradiation on Toxicity and Survival in Stage IIIA/IIIB Non-Small Cell Lung Cancer (NSCLC) Patients Treated with Chemoradiotherapy: Results from this aim will help to define critical anatomic cardiac structures and inform the dose constraints to be used on future lung and other trials where the heart is in the area of the radiation treatment field.

Aim 5: Evaluation of Hormone Therapy Length on Outcome for Intermediate Risk Prostate Cancer Patients: Results from this aim may lead to a trial to definitely evaluate radiotherapy with long-term hormones in the intermediate-risk prostate cancer patient population.

Aim 6: Evaluation of Changes in Serum Testosterone Levels in Prostate Cancer Patients Treated with Radiotherapy Alone: Results from this aim may lead to improvements in the amount of scatter radiation to the testes. This data may also serve as a control group for a future project to evaluate associations between radiotherapy modality and serum testosterone changes.

Summary of Research Completed

Aim 1: No progress to report for this period.

Aim 2: Discussions on the full statistical analysis plan have begun.

Aim 3: Statistical analyses were performed and an abstract was submitted to and presented at the 2015 Annual ASCO Meeting. The results are summarized below.

The effect of advanced age on outcome for single agent external beam radiation therapy (XRT) and combined modality therapy in locally advanced head and neck cancer (LA-HNC) is not well defined. This analysis will evaluate this question using several RTOG LA-HNC trials.

The effect of age (<70 vs >70 years) on survival and toxicity was examined in LA-HNC patients (pts) enrolled on three large Phase III trials: RTOG 9003 testing 3 altered fractionation (fx) XRT schedules vs standard daily XRT (SFX); RTOG 0129, comparing concurrent SFX + cisplatin (DDP) to accelerated fx with concomitant boost XRT (AFX-C) + DDP; and RTOG 0522, testing AFX-C + concurrent DDP +/- cetuximab. Cox regression analysis was used to evaluate the association between overall survival and age, as well as other covariates. Toxicities were evaluated using Chi-squared tests.

A total of 2688 patients that were eligible for analysis on the above mentioned trials were included in this analysis. The median follow-up for surviving patients was 5.2 years (range 0.01 to 20.3) overall; 14.1 years in RTOG 9003, 7.9 years in RTOG 0129, and 4.6 years in RTOG 0522. Only 11.5% (309/2688) of all patients were > 70 years, with 19.2% (207/1076), 6.7% (48/721), and 6.7% (54/891) in RTOG 9003, 0129, and 0522 respectively. Patients >70 years

were associated with being female with poorer performance status, heavier smoking history, and biomarker p16 negative status (p<0.001 each). There was an association with worse overall survival for patients over 70 years for the whole analysis, HR (95%CI), p-value 1.55 (1.35 – 1.77), <0.001; as well as for the individual trials: RTOG 9003: 1.34 (1.15 – 1.57), <0.001; RTOG 0129: 2.34 (1.68 – 3.26), <0.001; RTOG 0522: 2.45 (1.69 – 3.53), <0.001. Adjusting for covariates, age >70 had worse survival regardless of smoking or p16 status. Adverse effect of age >70 showed a trend for worse survival in p16 (+) pts (HR 2.07 vs. 1.30; interaction p=0.09), although there were only 34 patients in this subset. Maximum grade stomatitis and other toxicities were similar by age cohort and by treatment arms on RTOG 9003. In the DDP-based studies, the elderly experienced more grade 3-5 thrombocytopenia (p=0.02), anemia (p=0.03), nephrotoxicity (p=0.01) and possibly ototoxicity (p=0.06) but less mucositis (p=0.04).

Patients >70 years were under-represented in RTOG trials evaluating treatment for LA-HNC relative to their population overall. They were associated with worse overall survival compared to patients < 70 years; this was more apparent in combined modality, DDP-based trials, which featured heightened nephrotoxicity, myelosuppression, and ototoxicity. Delineation of causes of death and treatment compliance will provide insight into future trial design.

Aim 4: Additional contouring of the radiation treatment plans is ongoing, which is needed for additional statistical analyses.

Aim 5: The manuscript is in the process of being submitted to a peer-reviewed journal.

Aim 6: Following the initial analysis that was done, testosterone data became available for more patients than were included in the original analysis. In doing additional analyses in preparation for the manuscript submission, the entire statistical analysis was updated to include the additional patients. The results are summarized below.

Studies suggest radiotherapy (RT) may influence serum testosterone (ST) levels for patients treated for localized prostate cancer. This analysis evaluates data on testosterone changes for patients treated with RT alone on the Phase III prostate trial, RTOG 9408**.**

Patients enrolled on RTOG 9408 (T1b-T2b, PSA <20ng/ml) were randomized between RT alone and RT plus 4 months of total androgen ablation. RT consisted of either whole-pelvic RT to 46.8Gy plus a 19.8Gy prostate boost for a total dose of 66.6Gy (WPRT) or treatment to the prostate alone for a total dose of 68.4Gy (PORT). ST levels were investigated at: study enrollment; completion of RT; and first follow-up 3 months after completing RT. The Wilcoxon signed-rank test was used to compare change in pre- and post-treatment ST levels in patients randomized to the RT-alone arm.

2,028 patients were enrolled. 992 were randomized to receive RT alone. 917 (92.4%) of these patients had baseline ST values available and completed RT. Of these 917, immediate and 3-month post-RT testosterone levels were available for 447 and 373, respectively. Excluding 2 patients who received hormonal therapy off protocol after RT, 447and 371, respectively, were analyzed. Median pretreatment testosterone level for all 917 patients was 367 ng/dL (Q1-Q3=

279 to 466). For all patients, median change (5th and 95th percentile) in ST values at completion of RT and at 3-month follow up were -30.0ng/dL (p5-p95, -270.0–162.0; $p<0.001$) and -34.0ng/dL (p5-p95, -228.0-160.0; $p<0.01$), respectively showing a statistically significant associate for a decrease in ST level from baseline.

External-beam RT as delivered on the RTOG 9408 study was associated with a median 9.3% decline in ST at 3 months after RT. There was no significant difference in this decline based on whether patients received WPRT or PORT. These findings are consistent with most other series in the photon RT literature and suggest that low-dose scatter radiation outside of the beam path has a deleterious impact on testicular Leydig cell function. There is no evidence that these RT-associated changes in ST have been associated with an impact on clinical endpoints such as PSA control, erectile function, or quality of life, although that is a consideration for a future project.

Research Project 2: Project Title and Purpose

Community Learning of a Prediction Model for Treatment Outcome in Head and Neck Cancer Patients for Radiation Therapy Decision Support – Personalized medicine for head and neck cancer (HNC) is promising, but validated decision support systems are needed to make the promise a reality. A decision support system relies on a model to predict treatment outcome (e.g. survival, quality of life, toxicity). Such a model can be developed through a machine learning process using a well-organized database and query system that is designed for a community based rapid learning approach. This project aims to build such a model to guide head and neck radiotherapy treatment, and includes the development of an IT infrastructure for the Radiation Therapy Oncology Group (RTOG) to manage and deploy the clinical trial data needed for machine learning and building predictive models for radiotherapy treatment of HNC.

Anticipated Duration of Project

7/1/2013 – 12/31/2015

Project Overview

This project will test the hypothesis that it is feasible to build a decision support system to provide personalized radiotherapy treatment plans for head and neck cancer (HNC) patients. Three specific aims are proposed as follows:

Specific Aim 1: Build an IT infrastructure for machine learning. Clinical trial data used for machine learning requires full semantic interoperability so that the local data can be translated into a centralized database. The IT infrastructure also needs to support a community based rapid learning approach where routine patient data from many institutions in many countries is shared for learning. The design of the underlying technology will combine a local semantic interoperable environment with a distributed learning framework. When new patients (or new members) in the community become available an updated model can be learned.

Specific Aim 2: Modeling of survival in HNC based on our previous study. Utilizing an established machine learning system, a model that predicts the treatment outcome (including survival, toxicity, etc.) in HNC patients will be studied using the RTOG protocol 0522 clinical trial data. Classical approaches such as the logistic regression model as well as the so-called second-generation machine learning approaches such as Bayesian networks will be employed for modelling. The model performance is quantitatively evaluated.

Specific Aim 3: Extend the model by including more predictive parameters to improve model performance. Functional imaging procedures are employed more widely in cancer diagnosis and treatment. Large amounts of biological and molecular information become available as well with the advancement of sequencing technology. The project will explore these additional predictors in modeling to enhance the predictive performance of models.

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Expected Research Outcomes and Benefits

Extracting knowledge in the form of a prediction model can be used to change care delivery. Very specific questions like "what radiation dose should this head and neck cancer patient receive for an expected survival of X% at two years" can be answered. These are the type of questions that are being posed at the point of care.

The predictive models are built from a machine learning system that learns and shares knowledge while leaving the data behind the firewalls of the institutions. This system will be established as we complete Specific Aim 1 of this project. The important next step is to prove the rapid learning hypothesis that knowledge can be extracted from coordinated databases of routine care and clinical trial data sources. Using this system, learning can be done without data leaving the institute that holds the data.

The machine learning infrastructure can also be used to study other types of disease. Once deployed, the system can be leveraged in multiple research projects targeted at specific treatment modalities and specific cancers. Also, the technology is such that it can easily be applied outside of cancer. An open source solution, using semantic web technology and machine learning techniques, will boost the use of rapid learning in health care in the United States. The predictive models based on machine learning will be used to provide decision support in the personalized medicine era to give patients the best outcome: longer survival and better quality of life.

Summary of Research Completed

Specific Aim 1: While building the infrastructure, the team developed the following publication level abstract: "Validation of a rectal cancer outcome prediction model in routine Chinese patients."
Purpose/Objective: The risk of local recurrence, metastases and overall survival of locally advanced rectal cancer patients after preoperative chemoradiation and curative surgery can be estimated by prediction models and visualized using nomograms, which have been trained and validated in European clinical trial populations. This study aims to validate these prediction models in a routine clinical Chinese cohort.
Materials and Methods: From 2006 to 2012, the clinical data of 277 consecutive locally advanced rectal adenocarcinoma patients treated with preoperative chemoradiation and curative surgery from a single Chinese Cancer Center were retrospectively collected and used for external validation. Concordance index (C-index) and calibration curves were used to assess the performance of the previously developed prediction models in this routine clinical validation population.
Results: The C-index for the published prediction models was 0.72, 0.75 and 0.72 in predicting 2-year local recurrence (LR), distant metastases (DM) and overall survival (OS) in the Chinese population, respectively. Kaplan-Meier curves indicated good discriminating performance of local control; however, it wasn't successful at discriminating a low-risk and medium-risk group for distant control and overall survival. Calibration curves showed a trend of underestimation of local and distant control, as well as overall survival in the observed data compared with the model predicted one.
Conclusions: We externally validated three models for predicting 2-year LR, DM and OS of locally advanced rectal cancer patients who underwent preoperative chemoradiation and curative surgery with good discrimination in a single Chinese cohort; however, the model overestimated the local control rate compared to observations in the clinical cohort. Furthermore, validation in other clinical routine cohorts and optimization of the prediction model (including additional prognostic factors) will enhance model validity and enhance applicability for personalized treatment of locally advanced rectal cancer.

Specific Aim 2: The work on this aim resulted in the following publication format abstract:
Abstract 2: Artificial Neural Networks Applied to Overall Survival Prediction for Patients with Periampullary Carcinoma
Purpose: Artificial neural networks (ANN) can be used to discover complex relations within datasets to help with medical decision making. The purpose of this study was to develop an ANN

method to predict two-year overall survival of patients with peri-ampullary cancer (PAC) following resection.
Methods: Data was collected from 334 patients with PAC following resection treated in our institutional pancreatic tumor registry between 2006 and 2012. The dataset contains 14 variables including age, gender, T-stage, tumor differentiation, positive-lymph-node ratio, positive resection margins, chemotherapy, radiation therapy, and tumor histology.
After censoring for two-year survival analysis, 309 patients were left, of which 44 patients (~15%) were randomly selected to form the testing set. The remaining 265 cases were randomly divided into the training set (211 cases, ~80% of 265) and the validation set (54 cases, ~20% of 265) 20 times to build 20 ANN models. Each ANN had one hidden layer with 5 units. The 20 ANN models were ranked according to their concordance index (c-index) of prediction on validation sets. To further improve prediction, the top 10% of ANN models were selected, and their outputs averaged for prediction on the testing set.
Results: By random division, 44 cases in the testing set and the remaining 265 cases had approximately equal two-year survival rates, 36.4% and 35.5% respectively. The 20 ANN models, which were trained and validated on the 265 cases, yielded mean c-indexes as 0.59 and 0.63 on validation sets and the testing set, respectively. C-index was 0.72 when the two best ANN models (top 10%) were used in prediction on testing set. The c-index of Cox regression analysis was 0.63.
Conclusion: ANN improved survival prediction for patients with PAC. More patient data and further analysis of additional factors may be needed for a more robust model, which will help guide physicians in providing optimal post-operative care.

Specific Aim 3: No progress on this aim.

Research Project 3: Project Title and Purpose

Discovery of Plasma Biomarkers of Doxorubicin and Trastuzumab Induced Cardiotoxicity in Breast Cancer – The overall objective of this proposal is to discover novel circulating biomarkers using powerful proteomic profiling methods to identify patients at increased risk for doxorubicin and trastuzumab-induced cardiotoxicity, before conventional decreases in ejection fraction or heart failure are evident. The key targeted deliverables from this study are: 1) we will identify specific protein biomarkers indicative of early, subclinical cardiotoxicity; 2) we will gain insight into the mechanisms of doxorubicin and trastuzumab-induced cardiotoxicity, leading to new targeted therapies to prevent and treat this disease; and 3) we will build a multi-disciplinary collaboration for the study of cardiotoxicity biomarkers that we can expand to other cancer therapies.

Anticipated Duration of Project

1/1/2013 – 12/31/2016

Project Overview

Doxorubicin and trastuzumab (Herceptin®) are used widely in the treatment of breast cancer, are highly effective, and have led to important survival gains. However, these agents carry a substantial risk of cardiovascular morbidity and mortality. There is currently no adequate methodology to recognize patients at high risk for cardiac complications, prior to overt disease. The overall objective of this proposal is to discover novel circulating biomarkers using powerful proteomic profiling methods to identify patients at increased risk for both doxorubicin and trastuzumab-induced cardiotoxicity. Basic studies suggest potential mechanisms for cardiac dysfunction include oxidative stress, altered neuregulin/ErbB signaling, and anti-angiogenesis,[1-3] but the true relevance of these findings in humans and the precise mechanisms of cardiotoxicity remain to be elucidated. Furthermore, doxorubicin and trastuzumab cardiotoxicity are likely secondary to multiple altered and potentially differing pathways, and not one single mechanism. The broad working hypothesis of this proposal is that multiple circulating biomarkers, identified through discovery proteomics, will detect cancer therapy-induced cardiotoxicity in patients before conventional decreases in Left Ventricular Ejection Fraction (LVEF) or heart failure (HF) are evident. In breast cancer patients undergoing therapy with doxorubicin and trastuzumab, we will determine if patterns of change over time in protein markers differ between patients who experience cardiotoxicity and those who do not. In Aim 1, we will identify novel plasma biomarkers associated with cardiotoxicity in breast cancer patients treated with doxorubicin and trastuzumab. In Aim 2, we will verify the most promising biomarkers associated with doxorubicin and trastuzumab cardiotoxicity.

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Expected Research Outcomes and Benefits

The key outcomes from this novel study that will advance the field of cardio-oncology and improve the overall cardiovascular and oncology care of a growing cancer population are as follows: we will determine the utility of discovery plasma proteomics in identifying patients at risk for doxorubicin and trastuzumab-induced cardiotoxicity; and we will identify specific protein biomarkers whose changes in abundance levels are indicative of the early-stage development of cardiotoxicity. These two results alone will substantially advance the field of cancer therapy cardiotoxicity risk prediction. We will gain specific insights into the mechanisms of cancer therapy induced cardiotoxicity which has the potential to lead to new targeted therapies

to prevent and treat cardiotoxicity. This strong foundation of research has the potential to grow into multiple additional studies : 1) further verification and validation of the biomarkers identified herein; 2) pursuit of biologic mechanism leads; 3) development of new cardioprotective therapies indicated by the biologic leads; and 4) expansion of biomarker discovery and validation to additional cancers and cancer therapies. This work will serve as a critical launching pad to further build a cardio-oncology translational research program statewide and nationally, and strengthen collaborations between investigators at the University of Pennsylvania, Eastern Cooperative Oncology Group (ECOG), and American College of Radiology Imaging Network (ACRIN). It is anticipated, pending funding from other sources, a working group will be convened, comprised of cardiologists, oncologists, academic clinicians, and researchers, with the goals of developing strategies to enhance the detection of cardiotoxicity; innovative and cost-effective strategies for treatment and follow-up of cardiotoxicity; and recommendations for the management of cardiac comorbidities in cancer survivors. Health Research Funds from the Department of Health shall not be used to pay for expenses related to the work of this Committee.

Summary of Research Completed

This project was awarded on 4/15/2015. During this reporting period, efforts have focused on:

Aim 1: There are three relevant treatment groups that carry a substantive risk of cardiotoxicity: 1) Doxorubicin, without trastuzumab (Dox only), 2) Trastuzumab followed by doxorubicin (Trastuzumab-Dox), and 3) Doxorubicin followed by trastuzumab (Dox-Trastuzumab). In this project, we have focused our proteomics discovery experiments on cases and matched controls from the Dox only and the Dox-Trastuzumab groups. Work during this reporting period has focused on the Dox only group. Discovery within this latter group was being conducted by the Speicher laboratory under separate pilot funding which is no longer active. For the proteomics discovery efforts during the past year, we have made important progress in the intensive preparation of Dox only case and control plasma samples and analyses of these samples on a higher-sensitivity mass spectrometer (MS) with improved depth of analysis. We have also implemented an isobaric tagging method which enables samples to be multiplexed for increased throughput. Overall, we have identified approximately 600 proteins that can be quantitatively compared in all 3 cases and controls. Ongoing computational analyses are currently focused on identifying the best candidate biomarkers that demonstrate promising differences between cases and controls. For the Dox-Trastuzumab groups, we have been further validating potential candidate biomarkers that were identified in the initial 3-dimensional discovery analyses.

Aim 2: From the analyses, 520 proteins were identified and quantitatively compared in all cases and controls across these 30 samples. Statistical analyses are ongoing. Thus far, we have prioritized markers into the following categories: those in which case and control have significantly different intensities from the start of treatment and those that differ in protein levels at baseline and at/after the time of cardiotoxicity diagnosis. We have also explored differences in protein levels prior to and at/after the time of cardiotoxicity diagnosis.

There are a number of suggestive markers that differ according to case/control status. Table 1A contains a partial list of proteins which exhibited overall significant differences between case and control prior to any treatment (Student t-test p-value <0.05 and fold change >1.5). Table 1B lists proteins where the level of a given protein was significantly higher or lower at one or more pre-cardiotoxicity timepoints compared with after diagnosis of cardiotoxicity. Notably, two proteins, proteasome subunit alpha type-4 (PSMA4) and heat shock 70kDa protein 4 (HEL-S-5a) are significantly increased in all 3 cases when comparing pre-and post-toxicity plasma samples. Other biologically interesting markers identified in this group are paraoxonse-3 (PON3), which is an HDL-associated enzyme involved in lipid peroxidation, and superoxide dismustase (SOD2), a robust marker of reactive oxygen species. Table 1C denotes a comparison of protein levels at baseline and at the time of cardiotoxicity diagnosis among cases. Again, there are a number of potentially novel biologic markers identified, such as profilin-1(PFN1). Prolifin-1 is an actin binding molecule that has recently been implicated in cardiomyopathy development. Ongoing efforts are focused upon in-depth statistical analyses of the large number of candidate markers. Once the prioritized list of candidate markers has been developed, we will pursue specific assays (ELISAs or MRMs) to test these candidates across a broader range of samples.

Table 1A: Select Candidate Biomarkers Which Differ Significantly Between Patient and Control Prior to Treatment.

				Control Visits[a]													Patient Visits[b]																	
Gene names	Protein names	Fold Change[c]	p value[d]	C1v1	C1v2	C1v3	C1v5	C1v6	C2v1	C2v2	C2v4	C2v5	C2v6	C3v2	C3v3	C3v4	P1v1	P1v1d	P1v2	P1v3	P1v5	P1v6	P2v1	P2v1d	P2v2	P2v3	P2v4	P2v5	P2v6	P3v1	P3v1d	P3v2	P3v4
	Human MHC class III complement component C6	2.07	0.00	-2.33	-1.20	-2.43	-2.00	-2.47	-0.16	-0.51	-0.08	-0.04	-0.04	0.01	0.19	0.37	0.09	0.25	0.25	0.25	0.35	0.38	-1.32	-0.62	0.89	0.94	0.95	0.99	1.03	-0.16	-0.12	-0.08	-0.19
DKFZp686K18196	highly similar to Protein Tro alpha1 H,myeloma	2.04	0.00	-0.22	-1.29	-2.47	-3.22	-0.26	0.34	-0.43	0.06	-0.31	-0.33	0.18	0.11	-1.39	1.25	0.90	0.65	0.46	0.67	0.28	-0.45	0.09	-0.36	-0.70	-0.12	0.09	-0.12	1.10	0.56	0.83	0.25
MBL2	Mannose-binding protein C	1.85	0.01	-0.09	0.60	0.40	0.23	-0.24	-2.11	-1.47	-1.18	-1.55	-1.58	-0.77	-0.69	-0.78	1.10	1.08	1.17	1.83	1.18	0.72	0.03	0.19	-0.01	-0.31	-0.08	-0.38	-0.33	-0.92	-1.05	-0.63	-0.62
S100A8	Protein S100-A8	1.76	0.00	-0.14	0.30	1.27	-1.20	0.17	-0.06	0.12	-0.36	-0.92	-1.37	-1.05	0.13	-0.88	0.25	0.09	0.94	0.97	0.14	0.13	0.72	0.56	0.93	1.82	-0.20	-0.04	-0.63	0.18	0.37	1.14	1.20
LPA	Apolipoprotein(a)	1.72	0.04	-0.81	-1.20	-0.99	-0.95	-0.78	-0.82	-0.79	-0.37	-1.52	-1.57	0.03	0.42	0.06	0.73	0.77	0.87	-0.20	0.03	0.22	-0.57	-1.28	-1.13	-0.97	-1.15	-1.15	-1.58	1.47	1.47	1.62	2.07
CFHR4	Complement factor H-related protein 4	1.72	0.00	-0.12	0.59	0.44	0.47	0.64	-1.06	-0.21	-1.33	-1.21	-1.50	-0.43	-0.23	-0.05	0.65	0.62	0.98	0.79	1.01	0.35	0.37	0.77	0.40	1.08	0.60	0.47	0.48	-0.28	0.01	-0.30	0.01
S100A9	Protein S100-A9;Protein S100	1.61	0.00	-0.86	-0.15	0.36	-0.69	-0.14	-0.61	-0.46	-0.64	-0.93	-0.99	-1.15	-0.58	-0.89	-0.48	-0.36	0.31	0.54	-0.39	-0.01	0.46	0.46	0.28	0.82	-0.54	-0.24	-0.23	-0.06	-0.04	0.65	0.43
CFHR3	Complement factor H-related protein 3	1.51	0.00	-0.07	0.25	0.12	-0.08	-0.36	-0.75	-0.38	-0.23	-1.23	-1.25	-0.05	0.08	0.00	0.30	0.32	0.45	0.31	0.78	0.07	-0.22	-0.44	0.99	-0.35	1.25	0.88	0.42	0.20	0.16	0.07	-0.28
TRA1;HSP90B1	Endoplasmin	1.48	0.00	-0.43	-0.38	-0.47	-0.37	-0.51	-0.23	-0.03	-0.21	-0.30	-0.35	-0.50	-0.39	-0.36	0.37	0.33	0.28	0.47	0.51	0.39	0.48	0.52	0.13	-0.02	0.16	0.16	0.07	-0.16	-0.17	0.00	0.25
LTA4H	Leukotriene A-4 hydrolase;Leukotriene A(4) hydrolase	1.47	0.00	-0.10	0.12	-0.05	-0.09	0.03	-0.20	-0.18	-0.41	-0.37	-0.56	-0.44	-0.28	-0.31	1.27	1.23	0.60	0.96	1.22	0.05	0.49	0.40	-0.09	0.05	0.08	0.01	-0.11	-0.22	-0.05	-0.23	-0.02
SERPINA11	Serpin A11	1.46	0.00	-0.20	-0.22	-0.40	-0.30	-0.61	-0.29	-0.41	-0.46	-0.31	-0.46	0.07	0.15	-0.01	0.08	0.25	0.41	0.06	0.16	0.19	0.68	0.75	0.29	0.48	0.55	0.24	0.46	-0.05	-0.12	0.42	-0.06
CORO1A	Coronin-1A;Coronin	1.38	0.01	-0.42	0.22	0.41	-0.19	-0.70	-0.49	0.27	-0.44	-0.73	-0.71	-0.65	-0.46	-0.52	0.07	-0.15	0.32	0.54	0.18	-0.49	-0.34	0.14	0.09	0.50	0.13	0.27	-0.70	-0.19	-0.20	0.37	1.67
FUCA1	Tissue alpha-L-fucosidase	1.36	0.01	-0.83	-0.49	-1.11	-1.05	-0.86	0.18	0.56	0.16	0.44	0.26	-0.17	-0.10	-0.10	0.33	0.30	0.46	0.80	0.42	0.30	0.21	0.23	0.15	0.21	0.06	-0.03	0.20	-0.15	-0.14	0.15	-0.01
SFTPB	Pulmonary surfactant-associated protein B	1.34	0.04	-0.30	-0.03	-0.39	-0.12	-0.01	-0.09	-0.14	-0.12	0.76	0.40	-0.12	-0.31	-0.17	0.89	0.86	0.79	0.79	1.72	0.23	-0.49	0.55	0.05	0.17	1.04	0.84	0.41	-0.38	-0.49	-0.47	-0.09
GSTO1	Glutathione S-transferase omega-1	1.34	0.00	-0.17	-0.50	0.27	-0.19	-0.54	-0.40	0.08	-0.38	-0.52	-0.67	-0.68	-0.40	-0.41	0.23	0.10	-0.19	0.29	0.26	-0.63	0.25	0.26	-0.04	-0.01	0.13	-0.02	0.01	-0.11	-0.37	0.03	1.11
PGD	6-phosphogluconate dehydrogenase, decarboxylating	1.32	0.03	-0.11	-0.05	0.85	-0.17	-0.79	-0.85	-0.02	-0.80	-0.68	-0.79	-0.93	-0.44	-0.75	0.15	0.13	0.18	0.75	0.06	-0.79	-0.09	-0.01	-0.15	0.22	-0.39	-0.35	-0.32	-0.56	-0.53	0.38	0.98
CR2	Complement receptor type 2	1.32	0.03	0.77	0.07	-0.38	0.60	0.57	0.08	-0.75	-0.61	0.07	-0.02	-0.60	-0.75	-0.23	0.33	0.39	-0.20	-0.49	0.22	0.32	0.09	0.98	0.03	-0.20	0.59	0.90	0.69	0.78	0.83	-0.17	0.08
F11	Coagulation factor XI;Coagulation factor XIa heavy chain;Coag	1.30	0.03	-0.80	-1.02	-0.63	-0.53	-0.52	0.38	0.17	0.29	0.24	0.29	-0.49	-0.37	-0.44	-0.55	-0.56	-0.28	-0.47	-0.11	-0.25	0.14	0.07	0.51	0.53	0.58	-0.14	0.52	0.76	0.65	0.29	0.20
SERPINA6	Corticosteroid-binding globulin	-1.30	0.00	0.24	0.25	-0.03	0.31	0.34	-0.07	-0.04	-0.21	0.65	0.66	-0.01	-0.06	0.05	-0.03	-0.06	0.05	0.02	-0.22	0.24	-0.06	-0.12	-0.25	-0.27	-0.37	-0.29	-0.03	-0.55	-0.54	-0.49	-0.65
PON1	Serum paraoxonase/arylesterase 1	-1.30	0.03	-0.06	-0.36	-0.32	0.21	0.20	0.34	0.68	0.49	0.81	0.96	-0.32	-0.36	-0.28	-0.43	-0.42	-0.49	-0.39	-0.48	0.45	0.28	0.30	0.05	0.17	-0.18	-0.17	0.41	-0.74	-0.66	-1.02	-0.52
C7	Complement component C7	-1.30	0.00	0.30	0.22	0.28	0.34	0.23	-0.02	-0.11	0.11	0.27	0.07	0.03	0.21	0.20	-0.41	-0.50	-0.45	-0.53	-0.31	-0.18	-0.52	-0.48	-0.13	-0.02	-0.10	0.18	0.18	-0.06	-0.04	-0.13	-0.16
SMPDL3A	Acid sphingomyelinase-like phosphodiesterase 3a	-1.33	0.02	-0.24	-0.09	-0.22	-0.13	-0.52	0.20	0.75	0.26	1.05	0.42	0.05	-0.01	-0.08	0.18	0.06	-0.30	0.06	-0.16	0.58	-0.90	-1.03	-0.92	-0.57	-1.00	-0.81	-0.41	0.04	-0.10	0.12	-0.01
	Immunglobulin heavy chain variable region	-1.37	0.04	0.68	0.15	0.02	0.55	0.31	0.29	-0.35	-0.42	-0.28	-0.04	0.93	0.44	0.66	0.51	0.79	0.70	0.15	-0.16	0.29	-1.39	-0.39	-0.53	-1.50	-0.46	0.02	-0.55	-0.12	-0.32	0.08	-1.01
	FL cDNA clone CS0DD006YL02 of Neuroblastoma of Homo sap	-1.39	0.01	0.41	-0.24	-0.35	0.37	0.30	0.51	-0.28	-0.41	-0.28	-0.10	1.29	0.87	1.13	0.32	0.36	0.17	-0.41	-0.42	-0.22	-0.59	-0.62	-0.41	-0.47	-0.20	0.14	-0.08	0.07	0.12	-1.02	-0.53
PCYOX1	Prenylcysteine oxidase 1	-1.43	0.00	0.51	0.34	0.17	0.31	0.46	0.21	0.36	0.14	0.58	0.53	-0.01	-0.04	-0.08	-0.20	-0.27	-0.34	-0.34	-0.36	-0.18	0.10	0.05	-0.32	-0.27	-0.40	-0.10	-0.27	-0.17	-0.20	-0.38	-0.54
FCGR3B	Low affinity immunoglobulin gamma Fc region receptor III-B	-2.79	0.00	-0.58	0.53	0.94	-1.12	-0.52	-0.32	0.86	1.17	-1.01	-0.75	0.72	1.05	1.06	-1.86	-3.42	-1.55	-1.47	-3.29	-3.12	-0.63	-1.43	-0.06	-0.15	-1.63	-1.22	-1.10	-0.82	-0.41	0.06	-0.40
	Ig kappa chain V-III region HAH	-1.55	0.00	0.13	-0.94	-0.70	0.22	0.34	0.76	0.05	-0.75	-0.49	-0.37	0.93	0.55	0.73	-0.18	-0.37	-0.33	-0.59	-0.51	-0.55	-0.24	-0.51	-1.42	-0.74	-1.28	-0.94	-0.50	-0.21	-0.06	-0.89	-0.86
CNDP1	Beta-Ala-His dipeptidase	-1.61	0.00	0.53	0.38	0.00	-0.03	0.50	0.46	0.24	0.62	0.70	0.85	0.24	0.11	0.18	-0.59	-0.60	-0.44	-0.50	-0.59	-1.09	0.14	0.19	-0.05	-0.09	-0.01	0.04	-0.37	-0.37	-0.36	-0.33	-0.37
IGHG4	Ig gamma-4 chain C region	-1.62	0.00	0.23	0.16	0.03	0.50	0.63	0.60	0.56	0.13	0.69	0.76	-0.48	-0.36	-0.47	0.04	-0.22	0.07	-0.15	-0.08	-0.09	-0.40	-0.25	-0.71	-0.59	-0.79	-0.59	-0.31	-0.53	-0.44	-1.16	-1.67
DKFZp686P15220	Putative uncharacterized protein	-1.63	0.00	0.18	-0.54	-0.51	0.04	-0.11	0.20	-0.53	-0.52	-0.05	0.06	1.08	0.77	0.87	0.00	0.07	-0.01	-0.04	-0.13	-0.06	-1.32	-1.68	-1.45	-1.34	-1.36	-1.10	-0.56	-0.13	-0.22	-0.84	-0.64
	HUMAN cDNA FLJ78387	-1.65	0.01	0.51	-0.88	-1.39	0.92	0.96	0.28	-0.99	-0.86	-0.35	-0.22	1.03	0.50	0.84	-0.21	-0.26	-0.67	-1.34	-0.43	0.17	0.11	0.29	-1.20	-1.84	-1.18	-0.44	-0.78	-0.57	-1.13	-1.52	-0.86
HRG	Histidine-rich glycoprotein	-1.81	0.02	-0.18	-0.24	0.28	0.15	0.19	0.84	0.66	0.74	1.15	1.40	-0.37	-0.46	-0.26	0.16	0.14	0.40	0.62	0.78	-0.43	-1.18	-0.85	-0.02	0.09	-0.02	-0.64	0.21	-2.14	-2.22	-1.79	-2.55

a. All control visits. C1, C2 : Control 1, Control 2, etc.; v1,v2: visit 1, visit 2, etc; v1d: visit 1 duplicate (indicates technical replicate). The numbers in each cell (**a,b**) represent each protein's ratio compared to the reference sample. Blue highlight indicates the protein intensity is increased compared to the reference and red highlight indicates a decrease compared to the reference.
b. All patient visits. P1, P2 : Patient 1, Patient 2, etc.; v1,v2: visit 1, visit 2, etc.; v1d: visit 1 duplicate (indicates technical replicate).
c. Fold change between the average of all patient toxicity visits and the average of control visits. Green highlight indicates fold change was >1.5-fold higher in patients prior to treatment. Red highlight indicates fold change was >1.5 fold decreased in patients.
d. P-value calculated from student's T-test. P values that are highlighted yellow are <0.05.

Table 1B: Select Candidate Biomarkers Significantly Different Within Patients Prior to and At/ After the Time of Cardiotoxicity Diagnosis.

		Patient 1 Pre-Tox[a]				Patient 1 Tox[b]		Patient 2 Pre-Tox[a]					Patient 2 Tox[b]		Patient 3 Pre-Tox[a]			Patient 3 Tox[b]	Probability[c]		
Gene names	Protein names	P1v1	P1v1d	P1v2	P1v3	P1v5	P1v6	P2v1	P2v1d	P2v2	P2v3	P2v4	P2v5	P2v6	P3v1	P3v1d	P3v2	P3v4	p value P1[d]	p value P2[e]	p value P3[f]
PSMA4	Proteasome subunit alpha type-4	0.08	-0.10	-0.17	-0.06	-0.44	-0.56	-0.11	-0.06	-0.15	0.15	-0.16	-0.37	-0.27	-0.29	-0.41	0.17	0.69	0.01	0.05	0.03
HEL-S-5a	Heat shock 70 kDa protein 4	0.14	0.21	-0.22	0.22	-0.30	-0.84	-0.03	-0.02	-0.11	0.00	0.03	-0.21	-0.15	0.08	-0.09	0.44	0.86	0.04	0.02	0.04
PLA2G7	Phospholipase A2, group VII	-0.33	-0.44	-0.39	-0.60	-0.96	-0.88	0.18	0.55	0.27	0.08	0.36	0.13	-0.43	-0.09	0.04	0.03	0.79	0.01	0.08	0.00
SOD2	Superoxide dismutase	0.37	0.36	0.25	0.28	0.13	0.06	-0.08	-0.67	-0.42	-0.31	-0.37	-0.20	-0.43	-0.43	-0.71	-0.28	0.16	0.01	0.74	0.03
	Complement C1R Component	-1.12	-0.52	-0.81	-1.76	-2.68	-2.75	0.48	0.49	0.34	0.22	0.20	0.09	0.37	0.72	0.82	0.83	0.62	0.01	0.42	0.03
NOTCH2	Neurogenic locus notch homolog protein 2	0.32	0.31	0.36	0.42	0.17	0.19	0.01	-0.20	0.24	0.07	0.28	0.43	0.32	0.23	0.03	0.07	0.36	0.01	0.10	0.05
PON3	Serum paraoxonase/lactonase 3	0.20	0.17	0.23	0.17	0.37	0.26	0.20	0.20	-0.25	-0.12	-0.48	-0.16	-0.20	-0.28	-0.28	-0.28	-0.62	0.04	0.71	0.00
PNP	Purine nucleoside phosphorylase	0.08	0.09	-0.37	0.12	-0.50	-1.07	0.45	0.03	-0.86	-0.36	-0.49	-0.15	0.41	-0.38	-0.28	-0.40	1.21	0.04	0.39	0.00
ITLN1	Intelectin-1	-0.13	-0.19	-0.09	-0.68	0.23	0.51	0.55	0.27	0.10	-0.15	0.26	-0.04	0.27	-0.48	-0.55	-0.51	-0.06	0.04	0.69	0.00
GPNMB	Transmembrane glycoprotein NMB	0.14	0.16	0.14	0.37	-0.12	0.00	0.31	0.07	-0.04	-0.21	-0.18	0.03	0.03	-0.24	-0.24	-0.25	-0.20	0.05	0.81	0.00
SELL	L-selectin	-0.18	-0.17	0.02	-0.33	-0.48	-0.57	-0.55	-0.53	0.61	0.36	-0.41	-0.06	-0.01	0.00	-0.04	0.04	-0.30	0.03	0.87	0.00
ERAP1	Endoplasmic reticulum aminopeptidase 1	0.44	0.34	0.35	0.33	0.23	0.01	0.62	0.64	-0.04	0.10	0.25	0.12	0.41	-0.35	-0.38	-0.29	-0.04	0.03	0.85	0.00
CLIC1	Chloride intracellular channel protein 1	-0.09	-0.59	-0.05	0.13	-0.74	-1.33	-0.51	-0.60	-0.91	-0.22	-0.21	0.59	-0.82	-0.60	-0.43	0.27	2.26	0.04	0.42	0.01
PSMB6	Proteasome subunit beta type-6	0.00	0.13	-0.28	-0.04	-0.44	-0.55	0.09	0.17	-0.20	0.07	-0.14	-0.34	-0.24	-0.49	-0.34	-0.11	0.51	0.03	0.06	0.01
PRKCSH	Glucosidase 2 subunit beta	-0.01	-0.12	-0.06	-0.07	0.03	0.04	0.56	0.58	0.16	-0.15	0.36	0.32	0.13	-0.30	-0.27	-0.10	0.24	0.05	0.75	0.01
COLEC11	Collectin-11	0.08	0.18	0.00	0.24	-0.11	-0.19	0.64	1.29	0.26	0.13	0.27	-0.08	0.61	-0.02	-0.12	-0.08	0.13	0.03	0.56	0.01
PSMA6	Proteasome subunit alpha type-6	0.15	0.14	-0.07	-0.07	-0.20	-0.44	0.43	0.15	-0.20	0.05	-0.09	-0.24	-0.11	-0.28	-0.21	0.06	0.54	0.04	0.24	0.01
F5	Coagulation factor V	-0.26	-0.37	-0.05	-0.03	0.19	0.39	-0.39	-0.39	-0.35	-0.26	-0.33	-0.27	0.04	-0.04	0.00	0.22	0.54	0.03	0.05	0.02
VH6DJ	VH6DJ protein (Fragment)	-0.52	-0.71	-0.86	-0.72	-1.12	-1.08	2.44	2.73	0.29	-0.30	-0.31	-0.12	-0.20	-0.23	-0.35	-0.69	-1.14	0.02	0.36	0.03
F9 p22	Coagulation factor IX	-0.20	-0.17	-0.08	-0.04	0.04	0.19	-0.08	-0.06	0.19	0.15	0.06	0.09	0.17	-0.01	-0.08	-0.10	-0.20	0.03	0.44	0.03
GSS	Glutathione synthetase	0.04	0.00	-0.08	0.03	-0.14	-0.37	0.25	0.21	-0.02	-0.01	-0.01	-0.25	0.00	-0.12	-0.11	0.11	0.32	0.04	0.15	0.04
A1BG	Alpha-1B-glycoprotein	0.06	0.02	0.04	0.03	-0.26	-0.12	0.31	0.37	0.24	0.19	0.19	0.15	-0.02	-0.25	-0.18	-0.02	-0.25	0.01	0.04	0.36
SHBG	Sex hormone-binding globulin	0.30	0.24	0.08	0.37	-0.20	-0.73	0.08	0.00	0.15	0.23	-0.04	-0.11	-0.45	-0.35	-0.32	0.05	-0.20	0.02	0.03	0.95
PODXL	Podocalyxin	0.25	0.28	0.25	0.16	0.07	0.10	0.13	-0.41	-0.09	-0.10	-0.06	0.25	0.32	0.46	0.01	0.21	0.14	0.02	0.04	0.63
CETP	Cholesteryl ester transfer protein	-0.49	-0.47	-0.35	-0.11	-0.54	-1.05	0.41	0.37	0.51	0.69	0.24	0.01	-0.03	-0.05	0.02	0.21	-0.47	0.10	0.02	0.01
LDHA	L-lactate dehydrogenase A chain	0.20	0.24	0.17	0.50	0.21	-0.93	0.15	0.21	0.00	0.17	0.07	-0.08	-0.58	-0.30	-0.25	0.23	0.82	0.16	0.03	0.02
BMP1	Metalloendopeptidase	-0.47	-0.23	-0.65	-0.44	-0.46	0.35	0.64	0.45	0.00	0.36	0.23	-0.01	-0.37	-0.26	-0.31	-0.21	-0.54	0.23	0.05	0.01
PRG4	Proteoglycan 4	-0.49	-0.53	-0.49	-0.48	-0.74	-0.20	-0.30	-0.14	0.12	-0.16	-0.17	0.24	0.55	0.09	0.01	-0.01	-0.27	0.89	0.01	0.00
CPN2	Carboxypeptidase N subunit 2	-0.07	-0.14	-0.07	-0.07	-0.23	0.05	0.17	0.17	0.23	0.21	0.10	0.25	0.40	-0.38	-0.38	-0.30	-0.54	0.97	0.04	0.01
ITGB1	Integrin beta-1	-0.01	-0.24	-0.11	-0.14	-0.15	-0.11	-0.43	-0.21	-0.05	-0.22	-0.05	0.17	0.22	-0.09	-0.09	-0.18	-0.48	0.98	0.02	0.00

a. All patient pre-toxicity visits. P1, P2: Patient 1, Patient 2, etc.; v1,v2: visit 1, visit 2, etc.; v1d: visit 1 duplicate (indicates technical replicate). The numbers in each cell (**a, b**) represent each protein's ratio compared to the reference sample. Blue highlight indicates the protein intensity is increased compared to the reference and red highlight indicates a decrease compared to the reference.

b. All patient visits post-toxicity diagnosis.

c. P-value calculated from student's T-test of individual patient post-toxicity visits versus their pre-toxicity visits. P values that are highlighted yellow are <0.05.

Table 1C: Select Candidate Biomarkers That Differ Significantly Between Patients at Baseline and After the Diagnosis of Cardiotoxicity.

		Fold Changes		All Control Visits[a]														Patient Baseline Visits[b]						Patient Toxicity Visit[c]			Probability	
Gene names	Protein Description	Patient Tox vs Baseline[d]	Patient Tox vs Avg Control[e]	C1v1	C1v2	C1v3	C1v5	C1v6	C2v1	C2v2	C2v4	C2v5	C2v6	C3v2	C3v3	C3v4	P1v1	P1v1d	P2v1	P2v1d	P3v1	P3v1d	P1v5	P2v5	P3v4	Patient Tox vs Baseline[f]	Patient Tox vs Avg Control[g]
PFN1	Profilin-1	2.87	2.66	-0.27	-0.22	-0.21	-0.83	-0.86	-0.40	1.34	-0.98	-1.48	-1.19	-0.63	-0.22	-1.05	-0.81	-0.67	-0.66	-0.63	-0.59	-0.51	-0.42	0.67	2.37	0.02	0.02
WDR1	WD repeat-containing protein 1	2.15	2.25	-0.27	0.24	0.04	-0.22	-0.92	-0.44	0.89	-1.00	-1.24	-0.98	-0.95	-0.36	-0.89	-0.29	-0.45	-0.49	-0.09	-0.56	-0.55	-0.38	0.47	2.01	0.05	0.02
CD9	CD9 antigen	2.67	2.09	-0.16	-0.11	0.00	-0.61	-0.80	-0.13	1.05	-0.79	-0.68	-0.57	-0.84	-0.09	-0.61	-0.78	-0.91	-1.10	-1.14	0.00	-0.18	-0.51	0.41	2.29	0.05	0.04
CORO1A	Coronin-1A;Coronin	1.77	2.07	-0.42	0.22	0.41	-0.19	-0.70	-0.49	0.27	-0.44	-0.73	-0.71	-0.65	-0.46	-0.52	0.07	-0.15	-0.34	0.14	-0.19	-0.20	0.18	0.27	1.67	0.04	0.00
FLNA	Filamin-A	1.96	1.94	-0.23	-0.08	0.29	-0.83	-0.36	-0.37	0.93	-0.56	-0.93	-0.84	-0.41	-0.37	-0.02	-0.24	-0.10	-0.11	-0.52	-0.51	-0.32	-0.19	0.46	1.74	0.04	0.02
ENO1	Alpha-enolase;Enolase	1.64	1.82	-0.43	0.11	0.46	-0.76	-0.72	-0.68	0.55	-0.91	-0.54	-0.48	-0.75	-0.41	-0.39	-0.02	-0.08	-0.23	-0.19	-0.43	-0.42	-0.05	0.14	1.37	0.05	0.02
CALR	Calreticulin	1.28	1.46	-0.20	-0.15	-0.27	-0.20	-0.12	-0.09	0.29	-0.13	-0.33	-0.25	-0.08	0.14	-0.07	-0.02	-0.03	0.19	0.14	0.13	0.04	0.48	0.07	0.75	0.04	0.00
PEBP4	Phosphatidylethanolamine-binding protein 4	1.24	1.33	0.40	0.16	0.17	0.30	0.29	-0.18	-0.19	-0.04	-0.49	-0.43	-0.05	-0.16	0.10	-0.03	0.05	0.15	-0.09	0.42	0.08	0.40	0.23	0.59	0.05	0.03
SERPINF2	Alpha-2-antiplasmin	-1.12	-1.10	0.07	0.08	-0.11	0.07	0.16	-0.01	0.17	-0.02	-0.07	0.01	0.14	0.01	0.07	0.12	0.12	0.06	0.03	0.05	0.03	-0.01	-0.14	-0.13	0.00	0.02
SERPINA4	Kallistatin	-1.12	-1.18	0.19	0.16	-0.04	0.25	0.16	-0.12	0.07	-0.15	0.01	0.05	0.04	-0.20	0.00	0.02	-0.04	-0.01	-0.06	-0.07	-0.08	-0.24	-0.25	-0.14	0.00	0.01
SERPINA10	Protein Z-dependent protease inhibitor	-1.15	-1.19	0.22	0.36	0.15	0.04	0.28	0.16	0.33	0.04	-0.07	0.11	0.36	0.42	0.21	0.28	0.12	0.10	0.18	0.13	0.12	-0.15	-0.12	0.12	0.02	0.02
CR1;CR1L	Complement receptor type 1	-1.27	-1.20	-0.09	-0.09	0.24	0.22	0.17	-0.14	0.30	0.52	-0.02	-0.03	0.11	0.12	0.47	0.15	0.19	0.25	0.07	0.30	0.32	-0.22	-0.09	-0.09	0.00	0.05
	Ig kappa chain V-I region Ni	-1.56	-1.50	0.56	-0.08	-0.32	0.80	0.94	0.10	0.28	-0.68	0.51	0.27	0.22	0.05	0.25	0.11	0.35	0.69	0.27	0.09	0.18	-0.35	-0.61	-0.13	0.01	0.04
	Ig kappa chain V-III region HAH	-1.42	-1.75	0.13	-0.94	-0.70	0.22	0.34	0.76	0.05	-0.75	-0.49	-0.37	0.93	0.55	0.73	-0.18	-0.37	-0.24	-0.51	-0.21	-0.06	-0.51	-0.94	-0.86	0.01	0.05

a. All control visits. C1, C2: Control 1, Control 2, etc.; v1,v2: visit 1, visit 2, etc.; v1d: visit 1 duplicate (indicates technical replicate). The numbers in each cell (**a, b, and c**) represent each protein's ratio compared to the reference sample. Blue highlight indicates the protein intensity is increased compared to the reference and red highlight indicates a decrease compared to the reference.

b. Patient visits at baseline, before any treatment. P1, P2: Patient 1, Patient 2, etc.; v1,v2: visit 1, visit 2, etc.; v1d: visit 1 duplicate (indicates technical replicate).

c. Patient visits at the point of cardiotoxicity diagnosis.

d. Fold change between the average of all patient toxicity visits and the average of patient baseline visits. Green highlight indicates fold change was >1.5-fold higher at the cardiotoxicity diagnosis. Red highlight indicates fold change was >1.5 fold decreased at the point of toxicity.

e. Fold change between the average of all patient toxicity visits and the average of all control visits. Green highlight indicates fold change was >1.5-fold increased in patients having toxicity. Red highlight indicates fold change was >1.5 fold lower in patients at the point of toxicity.

f. P-value calculated from student's T-test of patient toxicity visits versus patient baseline visits.

g. P-value calculated from student's T-test of patient toxicity visits versus all patient control visits.

P values that are highlighted yellow are <0.05.

Research Project 4: Project Title and Purpose

Novel Statistical Analysis and Evaluation Methods for Multiple Endpoints in Cancer Clinical Trials – Clinical trials provide critical evidence necessary to advance clinical development in cancer research. The increasing number of promising new interventions mandates the improvement in clinical trial design and analysis, such that we can a) better understand disease progression; b) address clinical interests more quickly and efficiently; and c) conserve and optimize resources by terminating unpromising trials early. To address these needs, we propose a series of methodological projects aimed at addressing current questions in multiple endpoints in cancer clinical trials. These projects encompass a range of needs and challenges that apply broadly to cancer clinical trials and clinical research in general.

Anticipated Duration of Project

1/1/2013 – 12/31/2016

Project Overview

Aim 1: *Assessment of correlation between PFS and OS based on a Weibull model*: Progression-free survival (PFS) has been used as a surrogate marker for overall survival (OS) in oncology clinical trials. Accurate estimation of correlations between the two endpoints is important for trial design and outcome modeling. In previous work, an exponential model was considered for this purpose. However, observed hazard rates are often non-constant across time. In this research we aim to establish a Weibull correlation model which can provide more realistic estimates for this important quantity.

Aim 2: *Estimating Hazard of Failure Over Time in Early Prostate Cancer* – Typically, time to event data is summarized using aggregate measures such as time to event functions (i.e., survival curves, cumulative incidence curves) or cumulative hazards. The hazard function, being a dynamic time-varying process, may reveal features of the failure pattern over time that may have both biologic and clinical implications. However, hazard functions present challenges in terms of estimation and interpretation. In prostate cancer specifically, there are numerous questions regarding an individual's risk of failures of different types (biochemical failure, frank clinical disease, prostate cancer death and competing cause death) that have bearing on clinical management, as well as on gaining a better understanding of the disease process. We will investigate and compare different recently developed hazard estimation methods, and apply these to practical questions in long-term follow-up after treatment for localized prostate cancer.

Aim 3: *Evaluation of PFS and OS based on a progressive multistate model*: In oncology clinical trials, PFS is often considered as a putative surrogate endpoint for OS due to its clinical relevance and correlation with OS. However, the high correlation between PFS and OS, as well as the improvement in PFS alone do not always translate into an improvement in OS directly, therefore systematic evaluation and appropriate statistical models for PFS and OS are needed to address this issue. We propose to investigate and identify factors that may influence statistical inference of OS with reference to that of PFS.

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Expected Research Outcomes and Benefits

Clinical trials are a critical component of cancer research to advance effective interventions to prolong the survival of patients and save lives, and it is only through systematic and comprehensive evaluation in a clinical trial setting that the risks and benefits of treatment options can be assessed. However, the process of cancer clinical research can be slower than expected and resources are limited, especially with the tremendous amount of information that needs to be collected. Meanwhile, for clarity and robustness, a single primary trial endpoint (outcome of interest) must be chosen. To improve the process of cancer clinical research, a better understanding of multiple types of failure endpoints (disease recurrence of different types, death from cancer, death from other causes, etc.) experienced after cancer treatment is needed. This may offer additional pivotal insights into treatment efficacy, as well as inform trial design and analysis. These observations also provide information on disease natural history over time. A more efficient analysis and treatment evaluation strategy making use of all this information could improve both knowledge acquisition and patient care, which may rely heavily on our knowledge of the relationship between the multiple endpoints observed sequentially in cancer clinical trials. We propose three areas of statistical methodology research that have immediate practical implications for cancer clinical trials. These novel statistical methods can more directly assess risks, benefits and effects on investigative therapeutic agents, and will increase the trial operational efficiency and produce more informative outcomes. These investigations will provide a concrete demonstration of the worth of these innovative concepts and advance knowledge in cancer research and treatment.

Summary of Research Completed

Aim 1: During the past year, we have worked on revising the manuscript after including a third clinical trial example and more simulation results. We tested the assumption of a common shape

parameter for the Weibull model in the three oncology trial examples. We also ran extensive simulations to show that our proposed model is robust under exponential, Weibull, (same or different shape parameters) and log-logistic, lognormal distributions. Our proposed method performs better than the exponential model for these scenarios.

Aim 2: In the past year, we have worked to revise the submitted manuscript, the content of which was described in detail in last year's progress report. The revision in response to all of the peer reviewer comments has been extensive, and the manuscript is nearly ready to be re-submitted to the journal *Bayesian Analysis*.

We also completed a second study that focused on the modeling of biochemical failure (i.e., prostate specific antigen (PSA) rise above a threshold level) in relation to the duration of androgen deprivation therapy. This work used the pruned multi-resolution hazard (PMRH) modeling approach described in the first manuscript to explore important clinical questions in localized prostate cancer. Specifically, the work addresses whether the effect of androgen deprivation, (AD) which is demonstrated to reduce risk for numerous outcomes including biochemical failure, is persistent over the long term, or diminishes after AD ceases. In the data analyzed for this study (RTOG 92-02), AD was administered for either 4 months before and during radiation therapy (+0 group) or for 24 additional months (+24 group). The latter group has superior outcomes with respect to frequency of - and time until - biochemical failure, local/regional and distant metastatic disease, and death from prostate cancer. Thus, while it is recognized that long-term AD offers benefits during and for some time after the treatment period, it is not known how long this benefit lasts.

Using the PMRH method, the dynamics of biochemical failure hazard was modeled over time out to 12 years (Fig 1.1). It was seen that the relative benefit (hazard ratio) does diminish between the groups but this is in part due to the decreased risk for the short AD group which escaped early failure, as a large peak of failures typically occurs as AD is stopped. Similarly in the long AD group, immediately after active therapy (24 months), there is no peak but rather a sustained constant rate in the hazard, followed by a decline in hazard, and a small advantage over short-term AD persists through 10-12 years. Thus, it does appear that the benefits of the additional months of AD therapy, while diminishing over time, remain persistent and non-negligible. This is important in that it suggests that failure in the longer AD duration group are not simply deferred but possibly avoided completely. On the other hand, for those patients who received short-term AD therapy and did not fail early or during the peak period of failures, their late term prognosis eventually becomes nearly as favorable as those who underwent long duration AD. Thus, until such patients can be prospectively identified, the long AD approach would seem to be preferred for all patients but with better predictive risk markers, long-term AD may be avoided for some patients.

A manuscript describing these findings was submitted for publication in May 2015 to the *Journal of the Royal Statistical Society*, Series A (Applications). Reviews are expected shortly.

Aim 3: Evaluation of PFS and OS based on a progressive multistate model

In the past year we have been working on the development and evaluation of a progressive multistate model for progression-free survival (PFS) and overall survival (OS), including computational simulation studies under various scenarios. In particular, we considered a situation in which the true mechanism is believed to be of the recurrent event type (i.e., progression and death are always ordered) but the observed data has the appearance of the so-called "semi-competing risks" data structure in that some deaths are recorded without progression. Cox regression models for both progression and death are imposed in a hierarchical way to specify the joint model distribution of progression and death, as well as their relationship with baseline covariates. The proposed model has the potential to simultaneously assess the covariate effects on progression and death, such that the analysis from the proposed model can be used in conjunction with the univariate analysis of OS (and/or PFS) to collectively provide additional insights on how a particular covariate prolongs progression and death. Monte Carlo simulations have been conducted to evaluate the finite sample properties of model parameters. Additional simulation studies are ongoing to assess the model performance compared with conventional approaches under various real-world situations to provide guidance on how the proposed model can be used to inform disease management. Selection and preparation of the dataset from the RTOG lung cancer portfolio are also ongoing.

Figure 1.1

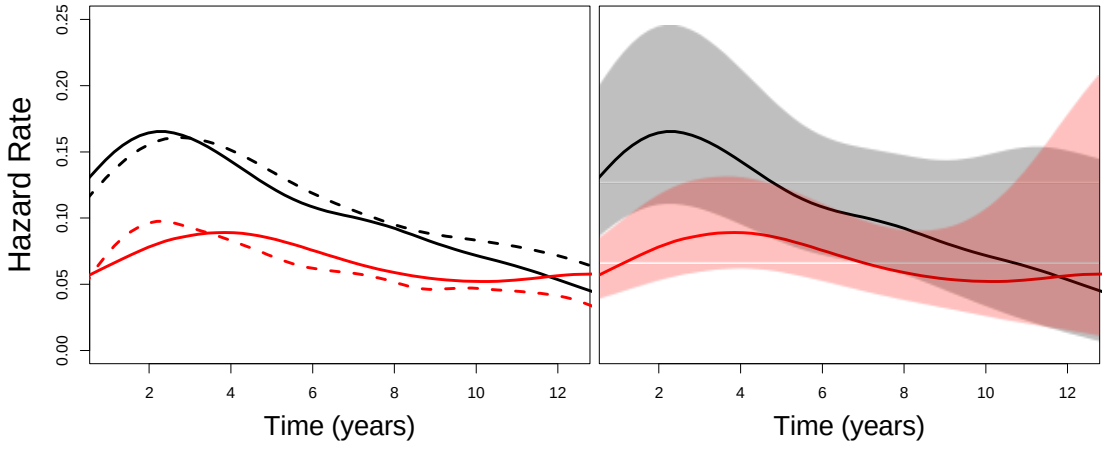

Figure 1.1: LEFT: Smoothed estimated hazard rates for the +24m AD group (red) compared to the +0m AD group (black). The hazard rates estimated under the non-proportional assumption are represented with solid lines, and the hazard rates estimated under the proportional assumption are represented with dashed lines (calculated as h0(t)exp(Bx)). While the estimated hazard for the +0m AD group is similar under both the proportional and non-proportional assumptions, the +24m hazard estimates have larger departures, with a later 2-year peak for the estimate from the proportional hazards model. RIGHT: Smoothed estimated hazard rates and 95% credible interval bounds for the +24m AD group (red) compared to the +0m AD therapy group (black). The intervals are slightly narrower for the +24m treatment group compared to the +0m group, although the credible intervals for the +24m estimated hazard rate become wider at the end of the end of the study where few failures are observed.