American College of Radiology

Annual Progress Report: 2010 Formula Grant

Reporting Period

July 1, 2014 – December 31, 2014

Formula Grant Overview

The American College of Radiology received $1,700,785 in formula funds for the grant award period January 1, 2011 through December 31, 2014. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Socio-demographic Factors, Workup, and Treatment for Cancer Patients in an Enhanced National Survey - The purpose of this project is to test hypotheses relating to quality of care and differences in socio-demographic factors for patients treated with radiation therapy for cancer of the breast, cervix, stomach, lung and prostate. Quality of care is defined by compliance with detailed clinical performance measures that include the patterns and sequence of particular types of surgery, radiation therapy, chemotherapy, hormonal therapy. Based on these findings we will make recommendations for improvement in the care of these groups of patients.

Duration of Project

1/1/2011 - 7/2/2012

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.pa.gov/MyRecords/Health-Research/CURE.

Research Project 2: Project Title and Purpose

Pennsylvania CT Dose Registry and Reduction Project - This project aims to study the effects of various interventions on radiation dose received by patients undergoing Computed Tomography (CT) scans at American College of Radiology Imaging Network – Pennsylvania (ACRIN PA) healthcare delivery sites in Pennsylvania. Dose data for all CT scans performed at the sites will be collected during a 6-month baseline period and analyzed to provide insight into practice variations resulting in different rates of exposure. Sites will then be randomized to one of

several strategies for education and implementation of CT dose reduction techniques during a 6-month intervention period, and dose data recorded for a one-year follow-up period. It is hypothesized that the average radiation dose delivered subsequent to the intervention will be lower than the dose delivered prior to the intervention.

Duration of Project

1/1/2011 - 12/31/2014

Project Overview

Four ACRIN Pennsylvania Network sites, which include community hospitals and outpatient clinics, performing CT in Pennsylvania will be identified to participate in the project. CT scan dose information will be collected from participating sites over a 6-month observational period. Sites will then be randomized to one of several dose reduction strategies and interventions will be implemented accordingly. Following the intervention, CT dose rate data will be collected for another year to determine how effective the intervention was in lowering dose.

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Expected Research Outcomes and Benefits

Knowledge of the current distribution of doses within this patient sample will allow more accurate analysis of and prediction of the amount of radiation received at CT and may provide background information for subsequent trials of larger scale which would enable more discrete analysis of variations (by manufacturer, scanner type, procedure, age, gender, etc.).

Evaluation of different dose reduction strategies will allow identification of best practices and implementation of strategies for reducing radiation exposure to Pennsylvanians, thereby reducing risk of radiation induced illnesses and cancers.

Summary of Research Completed

Specific Aim 1: To survey the distribution of radiation doses received at CT at select practice sites across Pennsylvania

Baseline data collection
During the July-December 2014 reporting period, baseline data collection was completed at three of the four sites: Pennsylvania State University-Hershey, University of Pennsylvania, and Geisinger Health System. The University of Pittsburgh and Bayer were not able to resolve their legal concerns regarding the software license, an impasse resulting in Pittsburgh's absence from the project.

Analysis
The radiation dose data collected during the baseline evaluation period totaled 84,299 exams (Figure 1) and were distributed among the following 15 common types of routine and advanced CT exams:

1. Unenhanced head CT
2. Head CT for sinus evaluation
3. CT angiography of the head and neck/circle of Willis/carotid arteries
4. Routine neck CT
5. Routine chest CT
6. Chest CT for pulmonary embolism evaluation
7. High resolution chest CT
8. Chest CT for lung nodule initial evaluation and follow up
9. Routine abdominal and pelvic CT
10. CT urography
11. CT angiography of the chest, abdomen, and pelvis for aortic dissection, aneurysm, or other aortic pathology and renal or mesenteric artery evaluation
12. Coronary CT
13. CT angiography of the abdominal aorta and iliofemoral runoff
14. CT guided interventions or biopsies
15. Cervical spine CT

We have begun analysis of the baseline acquisitions to test the hypotheses in our primary aim concerning variability and causes thereof in dose data across the different sites. As shown in Figure 2, there was a 2.5-fold difference in the head CT doses from the lowest to highest, ranging from an average of 1.7 millisieverts (mSv) to 4.5 mSv.

Variability amongst participating sites was much greater for scans of the chest, abdomen, and pelvis and cardiovascular scans. For example, Figure 3 shows average doses by site for chest CT examinations performed for evaluation of pulmonary embolism with average doses ranging from 2 to 21.6 mSv – a more than 10 fold-difference. If we restrict the analysis to sites with more than

50 exams, excluding some outliers on the low end, the range narrows somewhat – to 5-21.6 mSv, still a greater than four-fold difference.

Similar variability was found in abdomen/pelvis CT, as seen in Figure 4, with low dose sites in the 6-8 mSv range and high dose in the 24-26 mSv range. Even greater variation was seen in CT urogram doses, which ranged from 10 to greater than 70 mSv across sites. (Figure 5) Note that doses above 50 mSv for a single exam are in the range where there is reasonable evidence of a potential increase in the risk of cancer related to radiation exposure.
We have begun to analyze the reasons for such significant variability across sites and evaluated variability related to scanner manufacturer (Figure 6) and institution (Figure 7). There was an approximately two-fold difference in the lowest and highest average doses by manufacturer and by institution. Note that each institution roughly corresponds to an independently functioning group of radiologists. However, as there is also a dichotomy in scanner manufacturer between sites, i.e. sites with higher doses preferentially used scanners that had higher doses (GE and Toshiba) and sites that had lower doses preferentially used scanners that had lower doses (Siemens), it may not be possible to determine whether the observed differences are related to the equipment or protocol choices made by the radiologists at each institution. Other analyses being undertaken include stratifying dose by CT scanner generation (e.g. >64 slice, 64 slice, 16 slice, <16 slice) which may remove the confounding effect of the availability of newer technology across different sites.

Specific Aim 2: To evaluate the impact of various strategies for providing dose reduction education to sites performing CT in Pennsylvania.

Randomized Interventions
The second phase of the project required radiologist and technologist groups to be randomized to a particular educational intervention as indicated:

Technologist Group 1 Cohort: requires viewing of web-based manufacturer specific educational materials concerning radiation dose reduction methods available for the particular site's scanners

Technologist Group 2: Cohort: on-site training including presentation of recommended protocols for most common CT exams

Radiologist Group 1: Radiation dose report as provided by ACR's national dose registry
Radiologist Group 2: Monthly radiation report with more extensive analysis

The sites (hospitals and clinics) that were randomized and included in the study are shown in the following table:

Site		Location	
Hospital of the U of Pennsylvania		Philadelphia	
Radnor		Radnor	
Penn Presbyterian Med Ctr		Philadelphia	
Pennsylvania Hospital		Philadelphia	
Yardley/Langhorne		Yardley	
Valley Forge		Berwyn	
Chester County Hospital		West Chester	
Fern Hill		West Chester	
Kennett Square		Kennett Square	
Commons at Oakland		Exton	
Pennsylvania State U – Hershey Med Ctr		Hershey	
Geisinger Medical Center		Danville	
Geisinger Woodbine Lane		Danville	
Geisinger Wyoming Valley		Wilkes-Barre	
Geisinger Shamokin		Coal Township	
Geisinger Gray's Woods		Port Matilda	
Geisinger Lewiston		Lewiston	
Geisinger Mt. Pocono		Mt. Pocono	
Geisinger-Tunkhannock		Tunkhannock	
Susquehanna Valley Imaging - Lewisburg		Lewisburg	
Bloomsburg Hospital		Bloomsburg	

The on-site interventions were conducted during the reporting period while the web-based training continues to be collected and analyzed, using other funding sources.

Figure 1: Distribution of CT Examination Dose
Data Obtained During Baseline Period

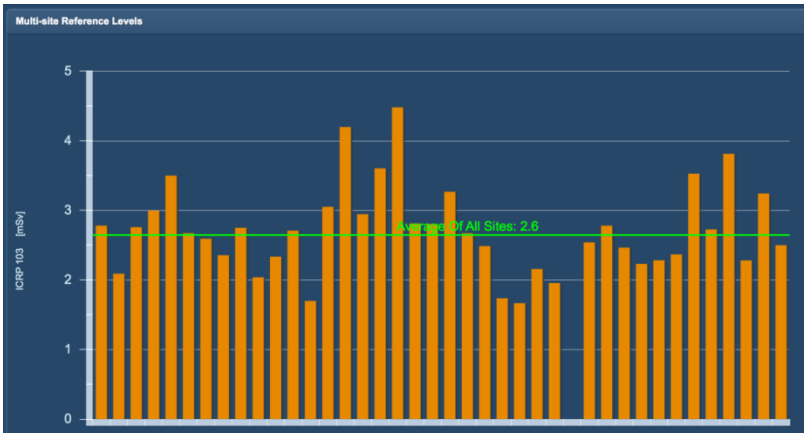

Figure 2: Average Dose of Head CT Examinations By Site

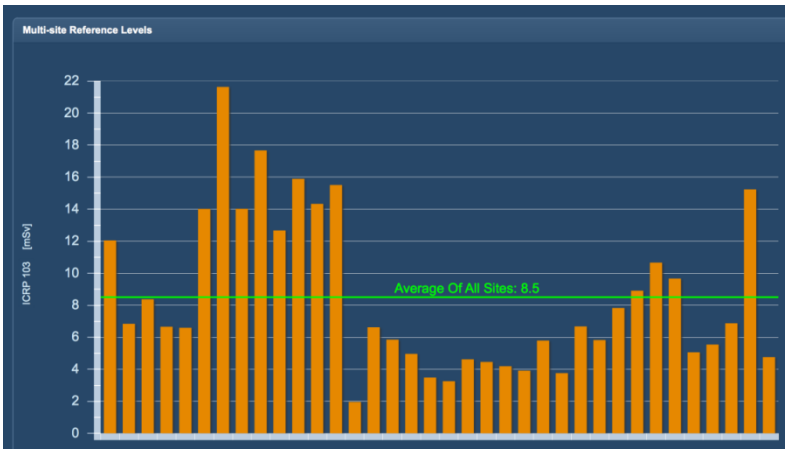

Figure 3: Variability in Average Dose for Pulmonary Embolism Chest CT exams

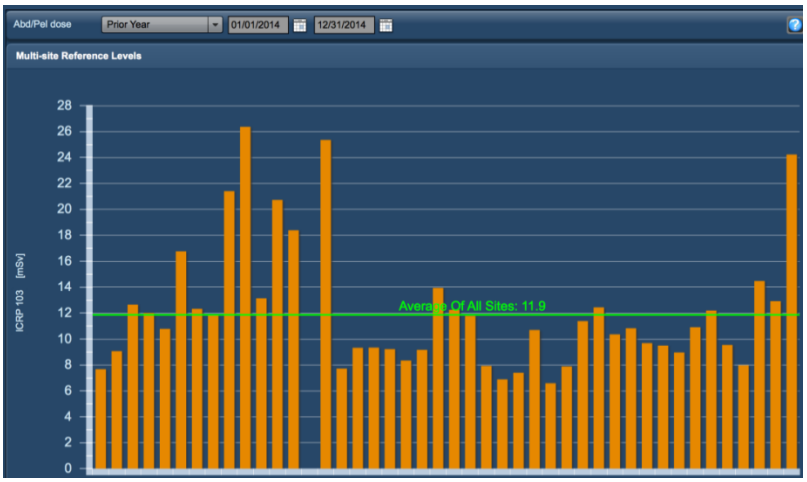

Figure 4: Variability in Abdomen/Pelvis CT dose across sites

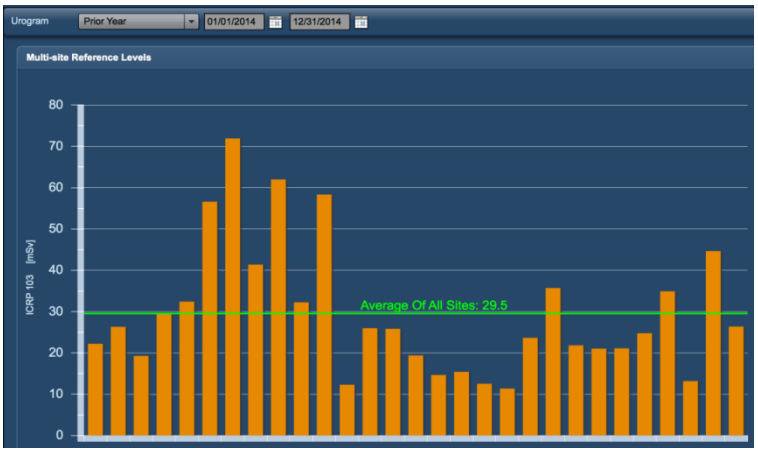

Figure 5: Variability in Doses for CT Urograms Across Participating Sites

Figure 6: Average doses during baseline evaluation by CT scanner manufacturer

Figure 7: Average doses during baseline evaluation period by institution.

Research Project 3: Project Title and Purpose

The Evaluation of Translational Research Program (TRP) Projects - The Radiation Therapy Oncology Group (RTOG), a National Cancer Institute funded multi-institutional clinical cooperative group has been collecting and banking biospecimens (biopsies, blood, urine, etc.) from patients enrolled on its clinical trials for decades. Often these specimens are collected without a pre-identified analysis – they are "banked" for future use. As technology and new biomarkers are developed, investigators request permission to use the specimens for research to identify new biomarkers or validate new procedures. These "secondary" analyses are not required by the original protocol, and may not be funded as part of that protocol. This project will allow for the investigation, including the statistical analysis, of five specified translational research program (TRP) projects.

Duration of Project

1/1/2011 - 12/31/2014

Project Overview

This project aims to use biomarkers and tissue specimens that have been collected in previous RTOG studies to advance current knowledge regarding the treatment and prognosis of cancer patients. The specific research objectives of this project relate to five TRP requests that will contribute to the overall project.

Aim 1: TRP 173: DPC-4 Status in pancreatic cancer patients: RTOG 9704, a Phase III trial of patients with resected pancreatic cancer, is a study that has resulted in several requests from investigators. For this project, the investigators will examine a patient's resected pancreatic cancer with intact DPC-4 to see if there's a local or incompetent metastatic phenotype as well as the correlation of DPC-4 loss with distant tumor recurrence using data collected in RTOG 9704. There will also be an investigation into DPC-4 status that is prognostic for overall survival.

Aim 2: TRP 165: Caveolin-1 and GSK3β in pancreatic cancer patients: Using data and samples collected in RTOG 9704, this project looks to determine whether Caveolin-1, GSK3β and related signaling molecules are prognostic biomarkers with regard to overall survival, disease-free survival, local failure-free survival and distant failure-free survival and correlate Cav-1 expression and pre-operative CA 19-9 levels.

Aim 3: TRP 167: Pharmacogenetic correlative science: The final project using data from RTOG 9704 has an overall goal to identify heritable, germline polymorphic markers that are prognostic and predictive of toxicity in pancreatic cancer patients. Efficacy and toxicity of previously identified putative germline genetic polymorphisms in this patient population will be examined.

Aim 4: TRP 169: Ribonucleotide reductase in cervix cancers: This project restricts its data to two cervical cancer trials: RTOG 0116 and 0128. The aim is to associate ribonucleotide reductase (RNR) M2 and p53R2 expression with survival.

Aim 5: TRP 91: Expression of receptors in bladder cancers: The final project utilizes multiple RTOG bladder sparing trials, particularly muscle-invasive bladder cancers treated with selective bladder preservation. The objective is to correlate the level of expression with the primary tumor site by immunohistochemical staining of VEGF and VEGF receptors, Flt-1 and Flk-1, with response, recurrence and survival.

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Expected Research Outcomes and Benefits

There are many benefits that will arise from these five TRP projects. The identification of new biomarkers will lead to changes in how RTOG designs studies, assigns protocol therapy, and

analyzes the results of its clinical trials. This research project will provide valuable information to aid future investigators in the design and conduct of radiation therapy-based oncology research.

Aim 1: TRP 173: DPC-4 Status in pancreatic cancer patients: This project may aid in the approval of a future RTOG proposal concerning a biologically directed technique for patients with intact DPC-4 and novel chemotherapy for patients with DPC loss.

Aim 2: TRP 165: Caveolin-1 and GSK3β in pancreatic cancer patients: Expanded knowledge of the current roles of Cav-1 and the GSK3β/β-catenin pathway obtained from TRP 165 will improve the prognosis of patients, discover new objectives for therapy and improve the development of trials for locally advanced pancreatic cancer.

Aim 3: TRP 167: Pharmacogenetic correlative science: TRP 167 will identify putative germline polymorphic prognostic and predictive markers to validate in this a large phase III study of pancreatic cancer.

Aim 4: TRP 169: Ribonucleotide reductase (RNR) in cervix cancers: TRP 169 will determine RNR inhibition following radiation as a therapeutic strategy as well as allow for future screens of candidate proteins identified in the DNA damage response pathway.

Aim 5: TRP 91: Expression of receptors in bladder cancers: There have been no reports concerning the correlation of cancer control with levels of VEGF and VEGF receptors in patients with muscle invading bladder cancer whose primary tumor has been treated by external irradiation or concurrent radiation and radiosensitizing chemotherapy, providing a rationale for TRP 91.

Summary of Research Completed

Aim 1: TRP 173: DPC-4 Status in pancreatic cancer patients: RTOG 9704 is a Phase III trial of patients with resected pancreatic cancer

Preliminary analyses were done for this aim. Comparisons of patient and tumor characteristics were conducted using the Chi-squared test. Overall survival was estimated using the Kaplan-Meier method and tested with the log-rank statistic. One-hundred and eight of the 538 eligible patients from the Phase III pancreas trial RTOG 9704 (title) we able to be evaluated for DPC-4. Missing data analyses were conducted and showed no significant differences in patient and tumor characteristics between patients with and without DPC-4 data, although there was a trend towards patients with a primary tumor location of head of the pancreas being associated with DPC-4 scores below the median. Additionally, there was not a significant difference in overall survival for patients with and without DPC-4 data ($HR_{(with/without)}$=1.07; 95% CI: 0.85, 1.36; p=0.57). The distribution of DPC-4 in nuclear AQUA_norm ranged from 773.2 to 5691.4 overall and full distribution statistics by treatment arm and overall are shown in the table below. For this preliminary analysis, a cut point of the median value, a common starting point for a cut point, was used. Based on this cut point, most of the patient and tumor characteristics were

balanced between the DPC-4 levels, with the exception of gender. Males were associated with higher levels of DPC-4 (p=0.0021). Two and 5-year overall survival and corresponding 95% CIs for patients with DPC-4 scores below the median were 28% (17%, 40%) and 11% (5%, 21%) respectively; and 43% (29%, 55%) and 25% (14%, 37%) for patients w/ DPC-4 scores above the median, respectively. The log-rank test showed a trend for an association with better overall survival for patients with DPC-4 scores above the median and a clear separation after 1 year, as shown in the figure below.

During this reporting period there was no additional progress on Aims 2, 3, 4 or 5.

Distribution of DPC-4 in Nuclear AQUA_Norm

	RT + 5-FU (n=55)		RT + Gemcitabine (n=53)		Total (n=108)	
	n	%	n	%	n	%
DPC-4 in Nuclear AQUA_Norm						
Median	1688.7		2013.2		1753.8	
Min - Max	773.2-5371.4		797.5-5691.4		773.2-5691.4	
Q1 - Q3	1441.1-2847.6		1269.2-3176.2		1273.3-3137.4	
< median (1753.8)	30	54.5	24	45.3	54	50.0
≥ median (1753.8)	25	45.5	29	54.7	54	50.0

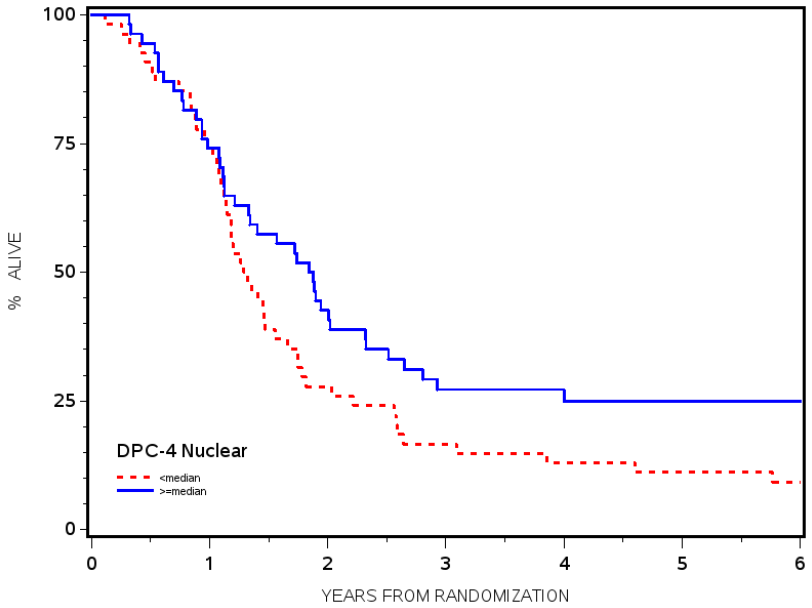
Characteristics of Patients by DPC-4 in Nuclear AQUA_Norm (n=108)

	<median (n=54)	≥ median (n=54)	p-value*
Age (years)			
Median	60	65	
Min - Max	37 - 80.96	35 - 80	
Gender			0.0021
Male	19 (35.2%)	35 (64.8%)	
Female	35 (64.8%)	19 (35.2%)	
Race			0.51
White	48 (88.9%)	50 (92.6%)	
Other	6 (11.1%)	4 (7.4%)	
Primary Location			0.81
Head	44 (81.5%)	43 (79.6%)	
Everything else	10 (18.5%)	11 (20.4%)	
KPS			1.00
80,70,60	20 (37.0%)	20 (37.0%)	

	<median (n=54)	≥ median (n=54)	p-value*
100,90	34 (63.0%)	34 (63.0%)	
T-Stage			0.83
T1,T2	16 (29.6%)	15 (27.8%)	
T3,T4	38 (70.4%)	39 (72.2%)	
N-Stage (surgical)			0.84
N0	18 (33.3%)	19 (35.2%)	
N1	36 (66.7%)	35 (64.8%)	
AJCC Stage			1.00
I,II	18 (33.3%)	18 (33.3%)	
III,IV	36 (66.7%)	36 (66.7%)	
Largest tumor dimension of primary			0.43
< 3 cm	24 (44.4%)	20 (37.0%)	
≥ 3 cm	30 (55.6%)	34 (63.0%)	
Primary tumor status			0.16
Complete resection/negative margins	26 (48.1%)	17 (31.5%)	
Complete resection/positive margins	17 (31.5%)	19 (35.2%)	
Complete resection/unknown margins	11 (20.4%)	18 (33.3%)	
RX			0.34
RT + 5-FU	30 (55.6%)	25 (46.3%)	
RT + Gemcitabine	24 (44.4%)	29 (53.7%)	

*p-value from Chi-square Test

Overall Survival by SMAD4 in Nuclear AQUA_Norm
(n=108)
Log-rank p-value = 0.098



Research Project 4: Project Title and Purpose

The Evaluation of Quality of Life (QOL) Endpoints in RTOG Studies - The Radiation Therapy Oncology Group (RTOG), a National Cancer Institute funded multi-institutional clinical cooperative group, conducts clinical trials with the goal of improving the survival and quality of life (QOL) of patients with cancer. RTOG has collected QOL data from both caregivers and patients for many of its trials. QOL outcomes are often not listed as a primary endpoint of the trial and therefore not funded by the original protocol. This project will allow for the evaluation of QOL of cancer patients receiving treatment in six specified RTOG protocols. The results of these assessments will provide valuable information regarding the treatments under study as well as the basis for the design of future studies.

Duration of Project

1/1/2011 - 12/31/2014

Project Overview

This research project aims to advance knowledge of quality of life in cancer patients. Due to the broad range of the effects of cancer and its treatment among the various disease sites, six specific research aims are proposed.

Aim 1: RTOG 9408: Patient's perception of quality of sexual function: This is a randomized Phase III trial investigating the effect of the combination of Zoladex and flutamide used prior to and during definitive radiation therapy on the patient's perception of quality of sexual function. A secondary objective is to determine the effect of the treatment on sexual function for patients in good prognosis with locally confined adenocarcinoma of the prostate.

Aim 2: RTOG 0247: Assessment of QOL changes from combined modality therapy: This is a randomized Phase II study evaluating neoadjuvant combined modality therapy for locally advanced rectal cancer. Changes in both overall and colorectal cancer-specific QOL concerns are of interest.

Aim 3: RTOG 0630: Exploring QOL in soft tissue sarcomas (STS): A Phase II trial, RTOG 0630 follows two cohorts of patients diagnosed with STS of the extremity on different image guided preoperative radiotherapy schedules This study explores late radiation morbidity, sexual and physical function and QOL.

Aim 4: RTOG 0129: Evaluation of radiation specific QOL: RTOG 0129 is a Phase III trial comparing two concurrent radiation and chemotherapy regimens for advanced head and neck squamous cell carcinomas. This study looks to evaluate whether there are differences in patient's QOL using a radiation specific QOL measure, performance status, health utilities and perception of side effects between each treatment arm.

Aim 5: RTOG 0522: Assessment of QOL, performance and health utilities: RTOG 0522 is a randomized Phase III trial designed to assess the impact of the addition of cetuximab to a concurrent radiation-cisplatin regimen for stage III and IV head and neck squamous cell carcinomas (HNSCC). QOL, performance and health utilities are measured up to 5 years post treatment, providing crucial long-term outcomes on this patient population.

Aim 6: RTOG 0244: Preventing xerostomia and improving QOL: This is a Phase II study of investigating the use of submandibular salivary gland transfer in head and neck cancer patients. Of main interest is the effectiveness of this treatment in preventing radiation-induced xerostomia as well as its impact, and possible improvement, on QOL since radiation therapy is one of the leading modalities for treating this population.

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Expected Research Outcomes and Benefits

For cancer patients QOL is a critical aspect to any treatment, especially if two treatments offer similar survival probabilities. QOL may then be the determining factor on choosing a treatment. Each of these aims will contribute to present knowledge regarding QOL in cancer patients. Better understanding of a patient's QOL will benefit the patients and aid investigators in the development and administration of new treatments.

Aim 1: RTOG 9408: Patient's perception of quality of sexual function: QOL is especially important in the realm of prostate cancer, where the treatment has a direct impact on the patient's sexual function. When investigating new treatments for prostate cancer, QOL must be taken into account as related to the patient's sexuality.

Aim 2: RTOG 0247: Assessment of QOL changes from combined modality therapy: Due to the lack of research on QOL in patients with rectal cancer, the QOL objectives in RTOG 0247 will provide information and estimates for the purposes of planning future Phase III trials.

Aim 3: RTOG 0630: Exploring QOL in soft tissue sarcomas (STS): In the disease site of sarcoma, current data is variable regarding QOL. Hence, the QOL component of this study will be a critical addition to current knowledge.

Aim 4:RTOG 0129: Evaluation of radiation specific QOL, Aim 5: RTOG 0522: Assessment of QOL, performance and health utilities & Aim 6: *RTOG 0244: Preventing xerostomia and improving QOL:* Treatment of cancer found in the head and neck, along with the cancer itself, can greatly impact a person's ability to eat, speak and socialize which adversely affects a patient's QOL. Therefore, studies like RTOG 0129, 0522 and 0244 need to incorporate a QOL component in order to measure how these difficulties affect a patient's life. Many other side effects of treatment for head and neck cancer have not been thoroughly studied so these three RTOG studies would provide further insight into this area where we have minimal data related to QOL.

Summary of Research Completed

Work on *Aims 1* and *2* was completed in a previous reporting period.

Aim 3: RTOG 0630: Exploring QOL in soft tissue sarcomas (STS)

<u>Purpose</u>
To assess QOL for patients with primary soft tissue sarcomas of the extremity.

Methods

Eighty-six patients not receiving chemotherapy were entered. Four tools, the Musculoskeletal Tumor Rating Scale (MSTS), the Toronto Extremity Salvage Score (TESS), the Functional Assessment of Cancer Therapy-General (FACT-G), and the Sexual Adjustment Questionnaire (SAQ), were collected at 4 time points: pretreatment, and 1, 1.5, and 2 years from start of treatment. For the MSTS and TESS, mean scores were compared against the NCIC trial preoperative arm using a two-sample t-test. Trends over time were evaluated using the generalized linear mixed model adjusting for baseline covariates (age, gender, race, performance status, tumor location, T stage, and histology). Pearson correlation coefficients were calculated between each pair of tools at each time point.

Results

Six patients in Cohort B were ineligible per protocol criteria and 1 patient did not start protocol therapy leaving 79 analyzable patients. Sixty-two of 79 patients (78.5%) consented to participate in the QOL study. Results of the comparison with the NCIC Trial Preoperative Arm are located in Table 1. Of the 20 patients with MSTS scores at 2 years, only 2 (10.0%) had Grade 2+ late toxicity at 2 years. With and without adjustment for a single covariate, the mean MSTS scores are roughly 31 for patients with Grade 0-1 late toxicity and 25 for patients with Grade 2+ toxicity. None of the covariates approach significance at the 0.05 level. The 4 patients with Grade 2+ late toxicity had mean 2-year TESS scores 10-15 points lower than the 22 patients with Grade 0-1 late toxicity. Wound complication approaches significance ($p=0.10$) but no other covariates approached significance.

The least squares means are very stable from pretreatment to 2 years (31.34, 30.56, 30.76, and 30.89) for MSTS and (87.28, 87.16, 87.11, 85.25) TESS. For both tools, none of the changes from pretreatment are statistically significant and none of the covariates approached significance. The least squares means for the FACT-G increase over time with the change from pretreatment to 2 years nearly statistically significant (5.13 point increase, $p=0.07$), and none of the changes from pretreatment are statistically significant. The least squares means for the SAQ are higher at all 3 post-treatment time points relative to pretreatment (36.25 to 41.49 to 42.34 to 40.60) with the 1-year and 1.5-year changes from pretreatment statistically significant (1-year: 5.24 point increase, $p=0.02$; 1.5-year: 6.09 point increase, $p=0.005$) and a nearly significant change from pretreatment to 2 years (4.35 point increase, $p=0.08$, Table 6.5). Age ($p=0.008$), gender ($p=0.05$), and histology ($p=0.03$) are significantly associated with SAQ score. The only significant correlations at 2 years occurred between MSTS and TESS ($r=0.43$, $p=0.07$) and between MSTS and FACT-G ($r=0.63$, $p<0.001$).

Conclusion

At 2 years, neither MSTS nor TESS showed improvement over the NCIC preoperative arm. Grade 2+ toxicity at 2 years may be associated with poorer MSTS, TESS, and FACT-G relative to Grade 0-1 toxicity. FACT-G may improve over time and SAQ may get worse over time while MSTS and TESS are stable. TESS and FACT-G are highly correlated.

Aim 4: RTOG 0129: Evaluation of radiation specific QOL

Purpose

To analyze the quality of life (QOL) of head and neck cancer (HNC) patients treated on RTOG 0129 by treatment and p16 status, and to examine the association between QOL and survival.

Methods

Eligible patients completed the Performance Status Scale for the HN (PSS-HN), the Head and Neck Radiotherapy Questionnaire (HNRQ), and the Spitzer Quality of Life Index (SQLI) at 8 time points from pre-treatment to 5-years post-treatment.

Results

QOL was not different between the two arms by area under the curve (AUC) analyses. However, patients in the accelerated arm had a larger drop in scores than those in the standard arm from baseline for several parameters: HNRQ and SQLI for the last 2 weeks of treatment ($p=0.002$ and $p=0.003$), PSS-HN Diet at 3 months ($p=0.03$), SQLI at 3 years ($p=0.04$), and PSS-HN Speech and SQLI at 5 years ($p=0.01$ both).

At pre-treatment and after one year post-treatment, p16-positive oropharyngeal cancer (OPC) patients had better QOL than p16-negative patients. However, QOL decreased more significantly from pre-treatment to the last 2 weeks of treatment in the p16-positive group. Pre-treatment PSS-HN was a significant independent predictor of overall, progression-free, and locoregional failure but not distant metastasis.

Conclusion

The results indicated patients in the accelerated arm experienced worse QOL than those in the standard arm. p16-positive patients had better QOL at baseline and after one-year follow-up. Patients presenting with better baseline QOL scores had better survival.

Aim 5: RTOG 0522: Assessment of QOL, performance and health utilities

Purpose

To analyze the quality of life (QOL) of stage III-IV head and neck cancer (HNC) patients enrolled on a prospective randomized phase III trial, comparing radiation-cisplatin without (arm A) or with cetuximab (arm B).

Methods

Eligible HNC patients, with QOL study consent completed the Functional Assessment of Cancer Therapy-Head and Neck (FACT-HN), Performance Status Scale for HNC (PSS-HN), and EuroQol (EQ-5D) at baseline through to 5-years. QOL was compared between treatment arms and by p16 status in oropharyngeal cancer (OPC) patients. Pretreatment QOL scores were correlated with outcome. Minimum important differences (MID) in QOL between groups were defined as $\geq$5% change in score.

Results

Of 818 analyzable patients, a 3.2% difference in mean change from baseline to 1-year in FACT-HN-total score was found between treatment arms. Mean EQ-5D-index and PSS-HN scores were not significantly different between arms. In OPC, p16-positive patients had significantly higher baseline and 1-year scores for PSS-HN, FACT-HN-Total and FACT-physical, functional subscales, baseline and 2-years for EQ-5D-index compared to p16-negative patients. Higher pretreatment QOL for PSS-HN-diet and eating, FACT-HN and EQ-5D-index scores were associated with better overall (OS), progression-free (PFS) survival on multivariate analysis. Higher FACT-HN-Total, FACT-functional, physical subscale, and EQ-5D-index scores were associated with better OS, PFS in p16-positive OPC, but not for p16-negative and non-OPC patients.

Conclusion

There was no difference in QOL between treatment arms. P16-positive OPC patients have significantly higher QOL than p16-negative patients. Pretreatment QOL is a significant independent predictor of outcome in locally advanced HNC.

Aim 6: *RTOG 0244: Preventing xerostomia and improving QOL*

Purpose

To report the results of quality of life of phase II RTOG – 0244 multi-institutional study which used submandibular salivary gland transfer (SGT) procedure for the prevention of radiation (XRT) induced xerostomia.

Methods

Eligible patients had surgery for primary, neck dissection, SGT followed by XRT during which the transferred salivary gland was shielded. IMRT, amifostine, and pilocarpine were not allowed, but postoperative chemotherapy was allowed. A modified version of the University of Washington Head & Neck Symptom Scale (UWHNSS) was used in this study. There was not a separate QOL consent for this study, so results of all patients and the patients who were treated as "per protocol" are reported.

Results

At baseline, all 44 analyzable patients completed the UWHNSS. The compliance at 3, 6 & 12 months was 85.7%, 67.9% & 64.3% respectively. 16 were not treated as "per protocol". For all 44 patients, at 3 months, 51.7% patients had either normal amount or mild loss of saliva while for 28 patients who were treated as "per protocol", at 3 months 66.6% had either normal amount or mild loss of saliva. Median follow up is 2.68 years and none of the patients had submental recurrence.

Conclusions

For patients treated as "per protocol", 66.6% patients had prevention of XRT induced acute xerostomia. Submandibular salivary gland transfer procedure is useful in the prevention of radiation induced xerostomia.

Table 1
Comparison of MSTS & TESS with NCIC Trial Preoperative Arm at 2 Years

Tool	Cohort	n	Mean	Standard deviation	Standard error of the mean	p-value (t-test)
MSTS Total score	RTOG 0630 cohort B	20	30.7	4.66	1.04	0.6810
	NCIC preoperative arm	35	30.0	7.90	1.34	
TESS Total Score	RTOG 0630 cohort B	27	81.6	18.64	3.59	0.4076
	NCIC preoperative arm	34	85.4	17.10	2.93	

Research Project 5: Project Title and Purpose

Improving the Collection of Patient-Reported Quality of Life Data - Expansion of Web-based QOL Collection Strategy - The purpose of this project is to test a novel strategy to reduce missing quality of life (QOL) data in clinical studies. While QOL is recognized as a key endpoint that provides direct patient reported outcomes, missing QOL data is a critical problem that plagues many clinical trials. Unlike other endpoints, such as survival, QOL data cannot be collected retrospectively. Typically, QOL forms are filled out on "hard" (paper) copies. This project will collect QOL using a real-time, privacy-secure, user-friendly, web-based software system such that patients can conveniently fill out their QOL forms on-line. The study will involve head and neck cancer patients with the goal of improving compliance of QOL data collection in this challenging population.

Duration of Project

7/1/2011 - 12/31/2014

Project Overview

The broad research objective is to rigorously test a new approach for collecting patient-reported quality of life (QOL) data in clinical trials in order to significantly reduce the challenge of

missing QOL data. Patient-reported outcomes, such as QOL, are recognized as key endpoints in clinical trials. Yet, missing data is an ongoing problem that limits the clinical relevance of many QOL studies. One of the most common reasons for missing QOL forms in multi-institutional studies is "institutional error", which might be something as simple the staff neglecting to provide patients with the QOL instruments at the proper time point. Recently, a novel web-based privacy-secure software system has been developed that enables patients to fill out their QOL forms on-line at their own convenience and provides real-time reminders. A preliminary pilot study in prostate cancer patients showed that this strategy significantly improved the QOL compliance at 6 months.

The primary aim of this project is to test this new software system in a more challenging patient population, specifically patients with head and neck (H&N) cancers. While the small pilot study in about 50 prostate cancer patients was encouraging, this was a relatively healthy group of patients. A more relevant test of this strategy is to determine its benefit in a population of H&N cancer patients, who typically have more involved symptoms and QOL challenges. Moreover, the primary time point in this project will be extended out to one year, rather than six months. If this project demonstrates that this strategy for collecting QOL can be successful in this more difficult setting, this approach could then be extended to a much broader group of cancer patients across many clinical oncology trials.

The main QOL instrument in this study is the validated Functional Assessment of Cancer Therapy (FACT)-H&N form. This QOL instrument was used in RTOG 0522, a phase III trial testing the addition of cetuximab to chemoradiation in a similar patient population. Based on this study using paper forms, the QOL compliance rate in H&N studies was about 50% at one year. Using this novel software system, the hypothesis is that this compliance rate at one year will be significantly increased to >65% (a 30% relative increase). The statistical design using 95% power would require 138 patients to show this difference. The study also has a 3-month QOL time point which would also be analyzed as part of this project.

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Mitchell Machtay, MD – employed by Case Western University
Clement Gwede, MD – employed by Moffitt Cancer Center

Expected Research Outcomes and Benefits

The expected outcome of this research project is to demonstrate that a web-based technology can significantly improve compliance regarding quality of life (QOL) as measured by completions rates within a clinical oncology trial. More and more, QOL is appreciated as a critical endpoint in clinical oncology studies. Indeed, the potential benefit of an intensive treatment is often counterbalanced by the increased rate of side effects. The optimal way to accurately assess the impact of these side effects is to collect QOL information directly from the patients (i.e., patient-reported outcomes). However, QOL studies are typically limited due to missing data, which must be collected at the appropriate time points. This project will test a promising strategy to enable patients to conveniently fill out QOL forms on-line using privacy-secure software. A prior pilot study in prostate cancer suggests that this approach can help improve the QOL collection rate. The expected outcome of this project is to expand this finding to a more diverse and complex group of patients with head and neck cancer.

The benefits of this project are potentially far-reaching. QOL is collected in many clinical trials and this project could change how these studies are done to make them more relevant and beneficial. Currently, most studies use paper copies of QOL forms; however, patients sometimes do not receive these forms or forget to fill them out. This novel web-based technology makes this process user-friendly for patients and allows for real-time tracking, such that an upcoming QOL time point that might have otherwise gone missing can instead be captured with reminders before the appropriate time window closes. Thus, this project has the potential to dramatically change and improve how QOL studies are performed and monitored.

Summary of Research Completed

Specific Aim: To test the hypothesis that, using a novel real-time web-based technology, the quality of life (QOL) compliance rate at one year will be significantly increased from 50% (using the prior methodology of collecting QOL via paper forms) to >65% (i.e., a 30% relative increase) in a study of head and cancer patients.

A secondary aim is to compare the QOL compliance rates at a shorter time point of 3 months, as well as the degree to which the specific items on the QOL forms are actually completed.

During the past six months, further milestones were accomplished on this project. The Radiation Therapy Oncology Group (RTOG) study 0920, entitled “A Phase III Study Of Postoperative Intensity Modulated Radiation Therapy (IMRT) +/- Cetuximab For Locally-Advanced Resected Head And Neck Cancer ” was opened at more institutions, including the amendment allowing for patients to complete their quality of life (QOL) forms using the electronic web-based technology (called VisionTree Optimal Care or VTOC). This amendment to allow for the use of this HIPAA-compliant electronic system had to be approved by each individual Institutional Review Board (IRB). This process went well and this component of this study (with this amendment) has been approved and opened at many RTOG institutions across North America.

Over the past six months (compared to the prior year), there was a 30% relative increase in the number of patients who consented to complete their quality of life on this study using VisionTree Optimal Care (VTOC) (for a total number to date of 96 patients). This represents 41% of the patients who consented to participate in the QOL component of RTOG 0920. The pretreatment characteristics of these 96 patients who consented to use VTOC are as follows: The median age was 57 years, compared to 59 years among other patients in the study. 65 of the 96 patients (68%) were male (compared to 66% of the patients in the rest of the study). Similarly, the breakdown by race was also similar. 88 of the 96 patients (92%) were white compared to approximately 83% of the patients in the rest of the study. Of these 96 patients who consented to use VisionTree Optimal Care, 40 (41%) had an excellent Zubrod performance status of zero. This appeared somewhat lower than the rate of 48% of patients in the rest of the study who had a Zubrod performance status of zero. Overall, the smoking history was similar among patients who consented to use VTOC and the remaining patients. 34 of the 96 patients who consented to VTOC (35%) had never smoked, compared to 35% of patients in the rest of the study. 23% of the VisionTree Optimal Care patients had less than 20 pack year history of smoking, 18% had 20 to 40 pack year of smoking, and 18% had a greater than 40 pack year history of smoking. The respective percentages in the remaining patients were 15%, 27%, and 20% (which is not significantly different).

Overall, the pathologic staging using the American Joint Committee on Cancer staging system (AJCC) was also similar between patients who consented to use VisionTree Optimal Care (VTOC) and the remaining group. AJCC stages I, II, III, and IV among the VTOC patients was broken down as follows: 3%, 12%, 21%, and 62%, respectively. Among the remaining patients, the breakdown was: 4%, 15%, 18% and 59%, respectively. In both groups, the rate of perineural involvement was 90%. The breakdown by pathologic T-stage was also very similar. Among the patients who consented to VisionTree Optimal Care, the breakdown of T1, T2, T3 and T4 was 24%, 42%, 15% and 18%, respectively. In the remaining group, it was 19%, 40%, 13% and 24%, respectively. The breakdown by pathologic N-stage was as follows for N0, N1, and N2: for the VTOC group it was 30%, 18%, and 48%, respectively; in the remaining group it was 40%, 17%, and 42%, respectively. Thus, at this point in the study, the pretreatment characteristics for the patients who consented to use VisionTree Optimal Care (VTOC) were mostly as expected and overall comparable to the remaining group of patients in the study who did not choose to use VTOC.

There is, however, an important exception in this regard. Regarding swallowing problems, there was a significant difference between the patients who consented to use VisionTree Optimal Care and those that did not. In particular, 49 of the 96 patients who consented to VTOC (51%) had no swallowing problems versus 65% of the patients who did not consent to use VTOC (p-value = 0.03). Rather, 46% of the patients who consented to use VTOC had swallowing problems present prior to registering on this study versus only 32% of the patients who did not consent to VTOC. This may be an important difference in that patients who are more symptomatic may be less likely to be compliant with additional components of the study over time, such as quality of life.

At this time, 55 patients who consented to complete their quality of life on this study using VTOC passed the one year follow-up time point. Three of the 55 patients (5.5%) who had consented to use VisionTree Optimal Care had passed away. Of the remaining 52 patients who were eligible to complete the validated Functional Assessment of Cancer Therapy – Head and Neck (FACT-HN) quality of life (QOL) form at one year, 27 patients (52%) did so within the QOL time window (of +/- 2 months). Another 5 patients completed the QOL form outside of this time window (for a completion rate of 62%). None of the patients withdrew their consent prior to the 1-year time point period. Of the patients who did not complete the FACT-HN form at the 1-year time point, the following were the reasons provided for the lack of compliance: Eight patients (14.5%) did not do so due to institutional error. One patient (1.8%) did not do so due to patient refusal. Four patients (7%) could not be contacted and one patient (1.8%) did not complete the QOL form for "other reason". Of note, for six patients (11%), the QOL form was not received and no reason was stated.
The hypothesis for this project was that the compliance rate for the validated Functional Assessment of Cancer Therapy – Head and Neck form (FACT-HN) at one year would be significantly increased from the baseline level of 50% (using paper forms in a prior study, RTOG 0522) to 65% using the electronic web-based technology, VisionTree Optimal Care (a 30% relative increase). Of note, the statistical design using 95% power would require 130 patients to show this difference. Thus, at this point, it is too early to make a statistical assessment regarding this hypothesis as, thus far, 55 patients who consented to using VTOC have completed radiation therapy at least one year ago. The information thus far suggests a trend in the right direction as 62% of the patients have completed the FACT-HN form at one year. Of note, previously, there were 6 patients who did not complete the 1-year QOL form due to "institutional error" and now (6 months later) there are 8 patients. This suggests that there has been some improvement in this regard due to a learning curve in this study for using VisionTree Optimal Care.

In summary, the following important milestones were accomplished over the past 6 months for this project. More patients were able to consent to using the electronic web-based technology, VisionTree Optimal Care (VTOC), with the appropriate IRB approval (as part of the RTOG study 0920). Moreover, with the exception of swallowing symptoms, the pretreatment characteristics of the patients appear similar to what would be expected compared to other patients enrolled in this study thus far. Finally, the 1-year validated Functional Assessment of Cancer Therapy – Head and Neck (FACT-HN) QOL compliance rate is tracking in the range expected and, as planned, further follow-up is needed to assess the hypothesis of this project.

Research Project 6: Project Title and Purpose

Leveraging the Androgen Receptor Axis to Improve Treatment of Locally Advanced Prostate Cancer - Treatment of locally advanced prostate cancer remains a major clinical challenge. New studies in our laboratory indicate that the androgen receptor (AR) axis can be manipulated to enhance the response to radiotherapy. The goal of this project is to develop a means for optimizing combinatorial therapy for locally advanced prostate cancer. Multiple *in vitro* and *in vivo* approaches will be utilized so as to provide the foundation for new clinical trials.

Duration of Project

7/1/2012 - 12/31/2014

Project Overview

Prostate cancer is the second leading cause of death due to cancer for American men. The current non-surgical standard of care for locally advanced prostate cancer involves a combination of radiation therapy and hormone-based, androgen-deprivation therapy (ADT). While ADT is intended to suppress the androgen receptor (AR) function through depletion of ligand, new studies in our laboratory indicate that alternate or adjuvant means to more robustly suppress AR signaling are likely to be of significant therapeutic benefit.

Preliminary data suggest the ***hypothesis*** that consideration and manipulation of the AR axis can be leveraged to improve treatment of men with locally advanced prostate cancer. This project will:
(Aim 1) Define means of targeting AR-mediate mTOR activity and sensitize prostate cancers to radiotherapy,
(Aim 2) Delineate the impact of newly identified AR antagonists on radiotherapy response.

Both aims of the project will utilize *in vitro* and *in vivo* analyses of human tumors, and the impact of combination therapy will be monitored using markers of clinical progression (e.g., prostate specific antigen (PSA), kinetics), apoptotic indices, proliferative indices, and measures of tumor growth. From these studies, it is expected that the knowledge gained will provide the basis for new RTOG clinical trials designed to optimally suppress AR and improve prostate cancer patient survival after radiation therapy.

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

The standard of care for men with locally advanced prostate cancer utilizes a combination of androgen deprivation therapy (ADT) and radiotherapy. Our preliminary data indicate that

targeting the androgen receptor (AR) axis directly would be of significant clinical benefit, and can act in concert with ADT to improve treatment outcomes for men with locally-advanced disease. This project will examine this hypothesis as follows:

1. It has previously been shown that AR utilizes the mTOR-cyclin D1 axis to promote cancer cell proliferation and survival. New studies in the lab indicate that antagonizing mTOR activity using existing experimental therapeutics not only suppresses AR activity but also sensitizes prostate cancer cells to radiotherapy. The subproject described in Aim 1 will determine the efficacy of mTOR inhibitors in human prostate cancer cells and tumors as a means to improve the therapeutic response to radiotherapy.

2. New studies show that direct AR antagonists can act in concert with ADT to improve the cellular response to radiation therapy. Aim 2 of this project will assess the relevance of this concept under conditions associated with advanced disease.

The results from Aims 1 and 2 are expected to provide the foundation for new clinical trials designed to improve outcomes for men treated for locally advanced prostate cancer. If successful, the present project could lead to dramatic improvements in the clinical management of locally advanced prostate cancer.

Summary of Research Completed

Aim 1 Progress:
Completed as per last progress report.

Aim 2 Progress
We have submitted a publication which is currently in review for a major journal. Major findings are summarized below:

DNA-PK interacts with AR and is recruited to sites of AR action
The ability of AR to alter response to radiation is controlled by DNA-PK. Our observation that DNA-PK is induced by AR activity and functions as an AR coactivator in advanced PCas that are progressing despite anti-androgen therapy (castration-resistant prostate cancer, CRPC) provided a strong impetus for further interrogation of DNA-PK-mediated transcriptional regulation and utilization of CRPC as a platform for discovery related to DNA-PK function. PCas are dependent on AR activity for growth and progression, and therapeutics intended to suppress AR activity through ligand deprivation (ie, pharmacologic castration through feedback inhibition) are the first line of therapeutic intervention for metastatic disease. While effective, tumors ultimately recur, almost invariably through restoration of AR activity. Thus, discerning the impact of DNA-PK on AR function in advanced disease is of obvious translational relevance. Consistent with identification of *PRKDC* as an androgen-regulated gene in CRPC, hormone deprivation decreased DNA-PK activity and levels in CRPC (Fig. 1A). As such, experiments to assess the function of DNA-PK as a transcriptional regulator were performed in hormone-proficient conditions. As expected, chromatin immunoprecipitation (ChIP) analysis revealed AR occupancy at two well-characterized AR regulatory loci, the *KLK3/*PSA and *TMPRSS2*

enhancers (Fig. 1B, left), wherein DNA-PK was also detected (Fig. 1B, right), demonstrating that DNA-PK is present at multiple AR regulatory loci, and similar to observations in hormone therapy (HT)-sensitive cells. In response to DHT, AR was recruited to each site within 30 minutes, with maximum occupancy observed at 16 hrs post-treatment (Fig. 1C, left). In contrast, DNA-PK occupancy was delayed until 6 hrs post-treatment, with maximum occupancy observed at 16 hrs (Fig. 1C, right). Combined, these findings suggest that DNA-PK is recruited to sites of AR function in response to AR occupancy. The impact of DNA-PK recruitment was determined by monitoring *KLK3/*PSA and *TMPRSS2* transcript levels in parallel studies. While significant induction of both *KLK3/*PSA and *TMPRSS2* was observed by 3 hrs post-DHT (Fig. 1D), maximum induction was not observed until after peak recruitment of AR and DNA-PK to regulatory sites, suggesting that DNA-PK occupancy is likely required for robust AR activity. Co-immunoprecipitation analyses further revealed that AR and DNA-PK are found in complex, and that the interaction is not further enriched by exogenous DHT (Fig. 1E). The AR-DNA-PK interaction is not dependent on DNA binding, as pre-addition of ethidium bromide did not disrupt the complex (Fig. 1F). Further, DNA-PK activity is not required for this interaction, as shown by treatment with the specific DNA-PK inhibitor NU7441. By contrast, NU7441 decreased DHT-stimulated AR target gene expression, further supporting a coactivator role for DNA-PK in AR-mediated transcription. In sum, these findings reveal that DNA-PK binds AR and impacts transcriptional activation at sites of AR action.

DNA-PK is a selective effector of transcriptional networks

Though AR is a known oncogenic factor in PCa, influence of many other transcriptional drivers made it imperative to discern the overall global impact of DNA-PK in regulation of transcriptional networks and cellular outcomes. Gene expression analyses were performed in CRPC cells either depleted of DNA-PK or treated with a specific DNA-PK inhibitor (Fig. 2A, left); as shown, the si*PRKDC* pool suppressed DNA-PK expression, whereas the DNA-PK inhibitor had no effect on DNA-PK levels (Fig. 2A, right). Genes identified as up- or downregulated by more than 1.5 fold were selected for further analysis (Fig. 2B). For both manipulations, the number of genes downregulated far exceeded those that were upregulated, suggesting that DNA-PK primarily positively regulates transcriptional events but can also function as a negative regulator of gene expression. Comparison between the groups demonstrated that DNA-PK depletion results in overlapping but distinct effects as compared to enzymatic inhibition. To minimize any potential off-target effects of NU7441, subsequent analyses were primarily focused on transcriptional alterations induced by DNA-PK knockdown. Gene Set Enrichment Analysis (GSEA) and associated motif analysis revealed significant enrichment of genes regulated by MAZ, MYC and the known DNA-PK-interacting partner Sp1, validating the concept that DNA-PK modulates a select subset of transcriptional networks (Fig. 2C). Additionally, GSEA gene ontology (GO) analysis demonstrated that genes sensitive to DNA-PK are associated with distinct biological processes including transcription, DNA-dependent transcription, and regulation of gene expression, further supporting a role for DNA-PK in gene regulation (Fig. 2D). Combined, these findings begin to define the cellular consequence of DNA-PK mediated transcriptional regulation, and demonstrate that DNA-PK selectively governs transcriptional networks.

DNA-PK and AR cooperate to suppress UGT enzyme expression in CRPC

Numerous pathways associated with metabolic and hormone pathways of potential clinical impact in PCa were identified as upregulated by DNA-PK depletion, including steroid hormone biosynthesis, wherein marked upregulation of UGT glycosyltransferases was observed. UGT enzymes catalyze the transfer of glucuronic acid to small hydrophobic molecules (including androgens), facilitating metabolism and excretion. In the prostate, local inactivation of androgens occurs when DHT is directly modified by glucuronidation or is metabolized to 5α-androstane-3α-diol (3α-diol) and androsterone, which are then glucuronidated by UGT2B15 and UGT2B17, both of which were upregulated by DNA-PK depletion. Consistent with previous reports suggesting that these genes are also AR regulated, AR occupied the proximal promoters of both *UGT2B15* and *2B17*, and residence increased in cells upon DNA-PK depletion. Parallel analyses revealed DNA-PK co-occupancy, suggesting that negative regulation of *UGT2B15* and *2B17* expression by DNA-PK is direct. DNA-PK depletion resulted in increased *UGT2B15* and *2B17* expression in two independent CRPC models, underscoring the impact of DNA-PK on this pathway. This finding is of strong translational relevance, as UGT2B15 and 2B17 are being developed as prognostic markers and pharmacologic targets for PCa management, and the mechanisms of regulation are not well understood. Notably, UGT2B15 and 2B17 protein accumulation was also enhanced upon DNA-PK depletion; as such, the impact of DNA-PK depletion on free and glucuronidated-DHT (G-DHT) levels was quantified by high-performance liquid chromatography (HPLC). While cells depleted of DNA-PK showed a trend towards decreased overall levels of free DHT, this did not reach statistical significance, suggesting that elevated UGT2B15 and 2B17 is not sufficient to independently alter hormone metabolism. GSEA KEGG analysis in response to the DNA-PK inhibitor further confirmed the selective function of DNA-PK as a negative regulator of transcription. On balance, these findings are the first to identify gene networks that are negatively regulated by DNA-PK, and identify DNA-PK as a key modulator of the UGT enzyme cancer-associated pathway.

Figures

Figure 1

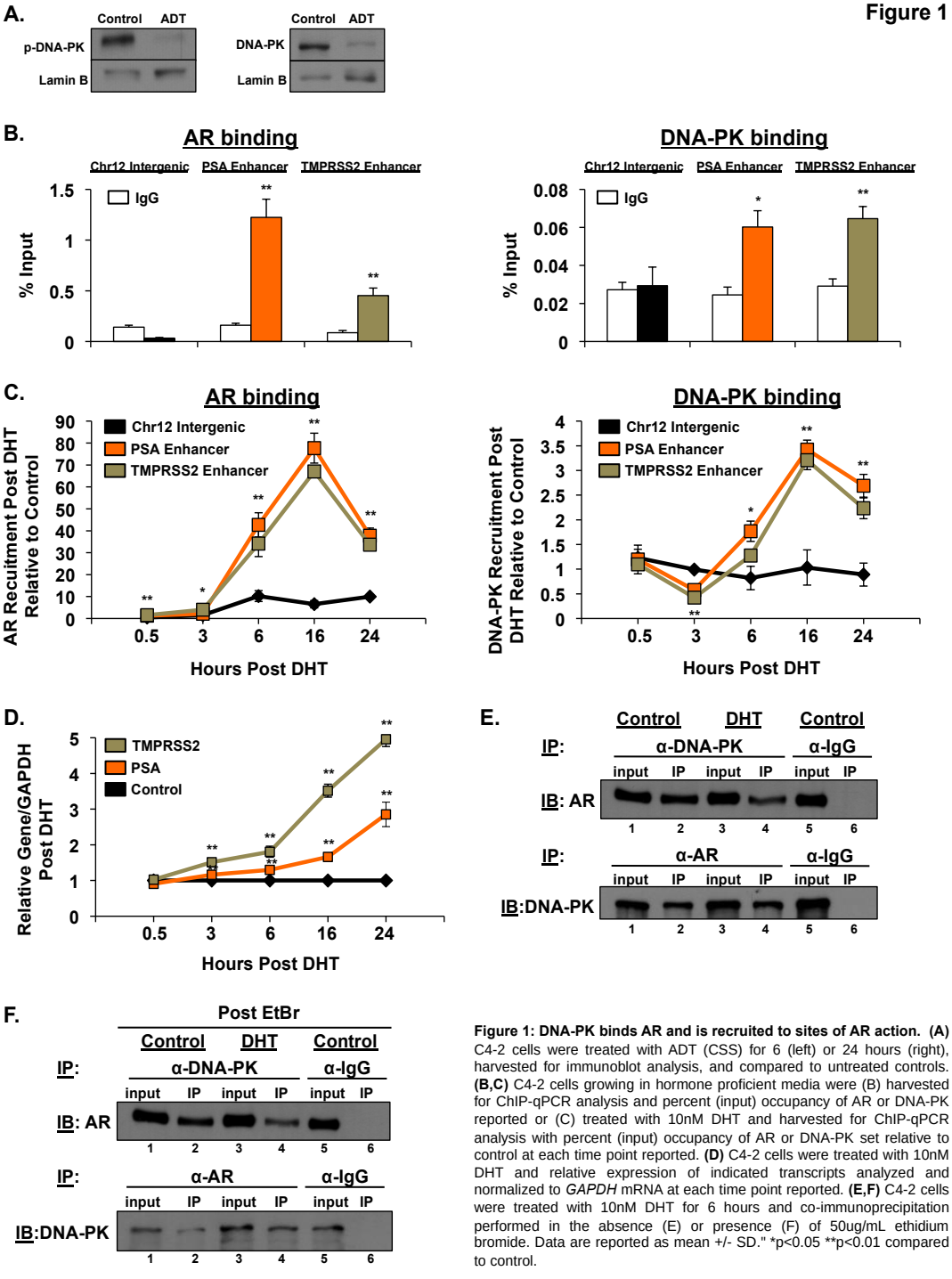

Figure 1: DNA-PK binds AR and is recruited to sites of AR action. (A) C4-2 cells were treated with ADT (CSS) for 6 (left) or 24 hours (right), harvested for immunoblot analysis, and compared to untreated controls. **(B,C)** C4-2 cells growing in hormone proficient media were (B) harvested for ChIP-qPCR analysis and percent (input) occupancy of AR or DNA-PK reported or (C) treated with 10nM DHT and harvested for ChIP-qPCR analysis with percent (input) occupancy of AR or DNA-PK set relative to control at each time point reported. **(D)** C4-2 cells were treated with 10nM DHT and relative expression of indicated transcripts analyzed and normalized to *GAPDH* mRNA at each time point reported. **(E,F)** C4-2 cells were treated with 10nM DHT for 6 hours and co-immunoprecipitation performed in the absence (E) or presence (F) of 50ug/mL ethidium bromide. Data are reported as mean +/- SD." *p<0.05 **p<0.01 compared to control.

Figure 2

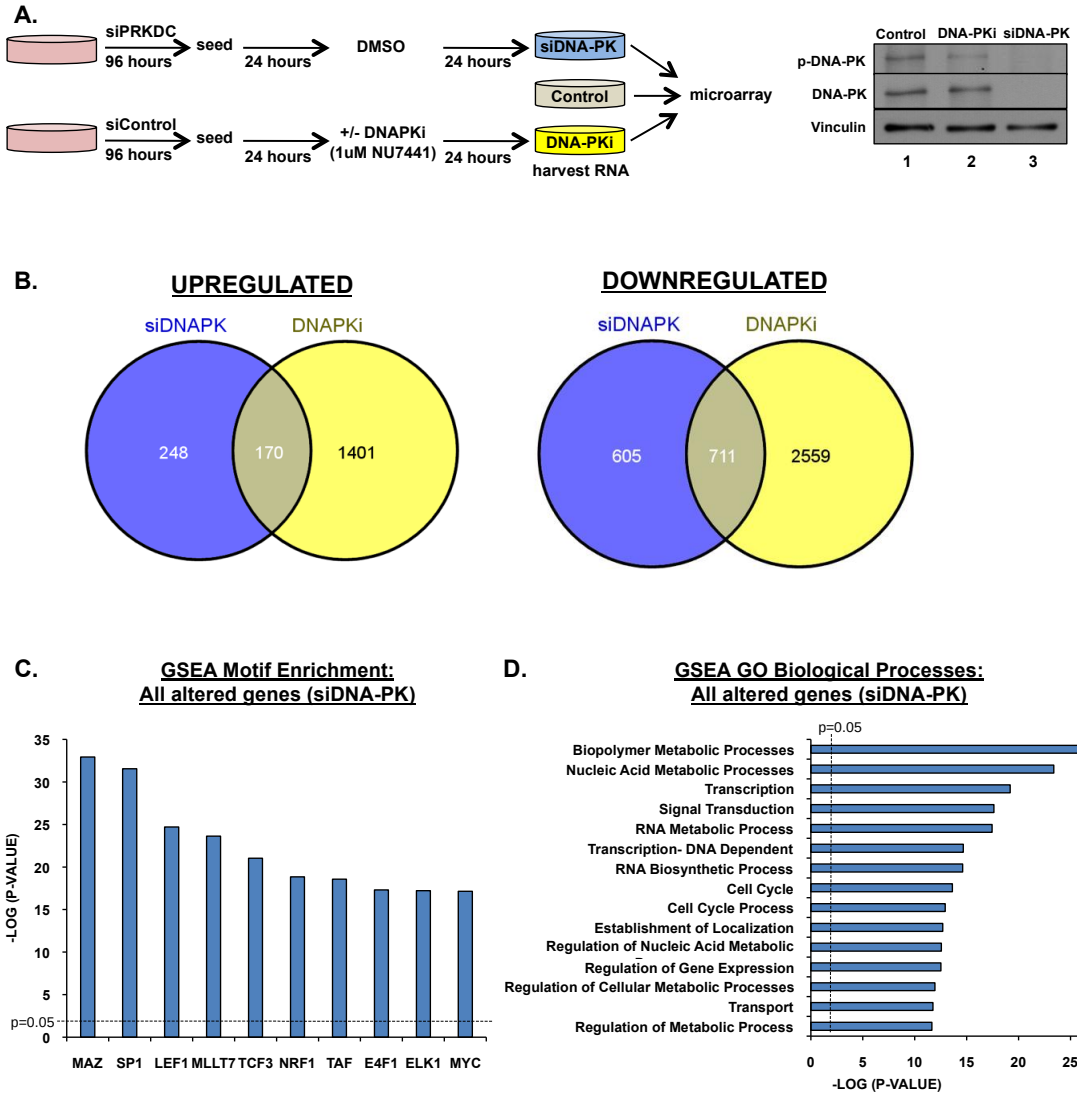

Figure 2: DNA-PK impacts global gene expression in CRPC. **(A)** RNA harvested from C4-2 cells depleted of DNA-PK or treated with 1uM NU7441 for 24 hours was analyzed by microarray analysis. **(B)** Genes identified by 1.5 fold cut off compared to untreated control. **(C,D)** GSEA analyses of all genes identified to be significantly altered after DNA-PK knockdown.