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The Influence of the Pretreatment Immune State on Response to Radiation Therapy in High Risk Prostate Cancer: A Validation Study from NRG/RTOG 0521

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Abstract

Purpose/Objectives: The immuno-inflammatory state has been shown to be associated with poor outcomes following radiation therapy (RT). We conducted an *a priori* designed validation study using serum specimens from RTOG 0521. It was hypothesized the pre-treatment inflammatory state would correlate with clinical outcomes.

Materials/Methods: Patients on RTOG 0521 had serum banked for biomarker validation. This study was designed to validate previous findings showing an association between elevations in C-Reactive Protein (CRP) and shorter biochemical disease free survival (bDFS). CRP levels were measured in pre-treatment samples. An exploratory panel of related cytokines were also measured including: monocyte chemotactic protein-1 (MCP-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), IL-1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A, IL-23, and tumor necrosis factor (TNFα). The primary endpoint examined was bDFS. Additional exploratory endpoints included overall survival, distant metastases, and toxicity events attributed to RT.

Results: 202 patients in RTOG/NRG 0521 had serum samples available. Median age was 66 years (48–83), 90% of patients were white. There was not an association between high sensitivity (hsCRP) and bDFS (adjusted hazard ratio [HR]=1.07 per one log increase in CRP, 95% CI: 0.83 – 1.38, p=0.60). In the exploratory, unplanned analysis, pre-treatment IL-10 was significantly associated with worse bDFS (adjusted HR=1.61 per log increase, p=0.0027) and distant metastases (HR=1.55 per log increase, p=0.028). The association of IL-10 with bDFS was maintained on a multiplicity adjustment. The exploratory analysis of pretreatment levels of IFN-γ, IL-1b, IL-2, IL-13, IL-23 were negatively associated with g 2 or higher pollakiuria (adjusted OR=0.64, 0.65, 0.71, 0.72, and 0.74, respectively, all $p<0.05$) and IL-6 was negatively associated with g 2 or higher ED (OR=0.62, p=0.027).

Conclusions: Pretreatment CRP is not associated with a poorer bDFS following RT. In a hypothesis generating analysis, higher baseline levels of IL-10 were associated with lower rates of bDFS. These findings require additional prospective evaluation.

Keywords

High risk prostate cancer; RTOG 0521; NRG 0521; immune system and prostate cancer response; CRP; IL-10; HS-CRP

Introduction:

The complexity of the host immune-inflammatory state is being increasingly demonstrated to influence the host response to oncologic therapy. The intersection of malignant progression and the host immune state represents an area of ongoing research that is in need of continued robust exploration. One such expanding area of investigation examines the intersection of radiotherapy (RT) induced cell kill and a host's pre-treatment immune-inflammatory state. It has been known for decades that the immune state of a patient may influence the response of a tumor to RT.[1] Such an interaction has been seen in prostate cancer, with growing evidence supporting the concept that chronic inflammation is involved in the regulation of cellular events associated with prostate carcinogenesis.[2,3] Moreover, malignancies developing in the setting of pro-inflammatory stimuli possess differences in the microenvironment, including vascular integrity, which may lead to differences in radiation responses.[4] Such data offers rationale for investigations specifically examining the host immune state and subsequent responses to RT.

Circulating cytokines represent critical drivers in the execution of a patient's immune and inflammatory response.[5] Such cytokines play important roles in the implementation of the host immune response. Cytokines serve to coordinate the complex immune interactions that occur during oncologic therapy. Furthermore, they enable the host immune system to respond to cancer progression or metastases.[6] Given the important role of inflammatory cytokines in the organization of the host immune response, examination of specific pretreatment cytokine levels presents an opportunity to understand a patient's specific capacity for immune coordination. Subsequent modulation of the host inflammatory state may also present an entirely novel chance to improve oncologic therapies and outcomes.

C-reactive protein (CRP) is a common and widely available acute phase reactant that is used extensively in the monitoring of numerous benign immune conditions.[7] In addition, CRP levels have been shown to correlate with prognosis in several malignancies including ovarian cancer[8], non-Hodgkins lymphoma[9], metastatic androgen refractory prostate cancer[10,11], endometrial cancer[12], melanoma[13], and metastatic renal cell carcinoma.[14] Two independent retrospective series in patients with non-metastatic prostate cancer have also correlated increases in CRP levels with worse oncologic outcomes following treatment specifically with RT.[15,16] Both retrospective series showed that increases in CRP levels correlate with poorer prostate cancer-specific outcomes, including biochemical failure. In addition, CRP levels have been validated in the ASCENT trial in metastatic prostate cancer to be associated with a poorer response to treatment with docetaxel chemotherapy, along with poorer overall survival.[10] In addition CRP has been shown to be associated with the development of toxicity in patients with breast cancer treated with RT.[17]

We designed a validation study of existing retrospective findings[15,16] using banked serum specimens from the completed clinical trial, NRG oncology/RTOG 0521. RTOG 0521 was a Phase III randomized clinical trial of androgen suppression and RT compared with androgen suppression, RT, and chemotherapy with docetaxel and prednisone for localized, high-risk prostate cancer.[18] Our central hypothesis in this validation study was that patients with increased levels of serum CRP before treatment would have lower rates

of disease-free survival following treatment for high risk prostate cancer. In addition to the planned validation of CRP levels, we also collected several pretreatment serum cytokine levels associated with the CRP inflammatory cascade to further characterize the interaction between more specific metrics of the pre-treatment inflammatory state and response to treatment with RT.

Materials and Methods:

Study Design, Hypotheses, and Patient Population

We hypothesized in this *a priori* designed validation study that high risk prostate cancer patients with elevated serum CRP levels would have worse prostate cancer specific outcomes when treated with RT, androgen deprivation therapy, +/− chemotherapy as compared to those patients with normal CRP levels. We further hypothesized that additional serum inflammatory cytokines correlate with prostate cancer responses to radiation. To test these hypotheses, banked specimens and clinical data from the phase III clinical trial RTOG 0521 were utilized. The patients included on the 0521 protocol were patients over the age of 18 with high-risk adenocarcinoma of the prostate. They were stratified in the parent study based upon four disease characteristic combinations consisting of group 1: Gleason ≥ 9, PSA ≤ 150, and any T-stage, group 2: Gleason 8, PSA < 20, and ≥ T2, group 3: Gleason 8, PSA ≥ 20–150, any T-stage, and group 4: Gleason 7, PSA ≥ 20–150, any T-stage. The primary objective of the parent study was to evaluate the role of adjuvant docetaxel chemotherapy in this patient group. The parent study, including primary outcome results, has been previously published.[18] This ancillary study was approved by the institutional review board (IRB) of the **** along with the National Cancer Institute (NCI).

Procedures and Cytokine Assay:

High-sensitivity (hs) CRP levels and standard CRP levels were measured in pre-treatment samples using a widely available, clinical grade assay in a clinical laboratory improvement amendments (CLIA) certified laboratory.[19] The assay used was manufactured by Roche Diagnostic (Indianapolis, IN). Specifically, this assay is an FDA approved particle enhanced immunoturbidimetric assay that is run on the Roche cobas c-system. This assay has a test code of 800720.

In addition to the above-mentioned CRP assay, an exploratory multiplexed array of fifteen serum immune-inflammatory cytokines was performed for each sample. These serum cytokines were carefully selected for their prior examination in either pre-clinical or clinical prostate cancer studies and/or overlap with CRP in the inflammatory mechanistic cascade. This was an exploratory aspect of the analysis. The multiplex cytokine assay was performed by Eve Technologies (Calgary, AB, Canada) by using the Bio-PlexTM 200 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and a Milliplex human cytokine kit (Millipore, St. Charles, MO, USA) according to their standard protocol. The measured cytokines consisted of monocyte chemotactic protein-1 (MCP-1), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), IL-1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17A, IL-23, and tumor necrosis factor (TNFα). The assay sensitivities of these markers range from 0.1 – 9.5 pg/mL. Individual analyte values and other assay details

are available on Eve Technologies' website (www.evetechnologies.com) or in the Milliplex protocol.[20] If an assay result was flagged as having sample saturation or a low sample volume, that sample was excluded from analysis.

Outcomes and Objectives:

The primary endpoint examined in this validation study was biochemical disease-free survival (bDFS), defined as the time from randomization to the development of biochemical failure (PSA >=2.0 ng/ml above the serum nadir or initiation of non-protocol hormone therapy), local progression, distant metastases, or death from prostate cancer, whichever occurred first. Patients who died from causes other than prostate cancer before having any of the above events were censored as of the time of death. Secondary endpoints were distant metastases (with death prior to distant metastasis treated as a competing risk), and overall survival. A tertiary objective included analysis of the association between the inflammatory biomarkers and five of the most frequent toxicity events attributed to RT in the RTOG 0521 trial, namely: pollakiuria ≥ grade 2, cystitis ≥ grade 2, diarrhea ≥ grade 2, erectile dysfunction (ED) ≥ grade 2 and ED ≥ grade 3, and proctitis ≥ grade 2.

Sample Size Calculation:

RTOG 0521 opened December 8, 2005 and closed on August 21, 2009 with a total of 612 patients enrolled of whom 563 were deemed eligible in the April 2015 analysis report. There were total of 116 PSA failures, 35 patients for whom non-protocol hormone therapy was initiated, 3 local failures, and 16 distant metastases recorded as first failure events. Assuming all prostate cancer deaths would have been preceded by one of these failures, we projected 170 bDFS events. Further assuming that baseline biospecimen collection is independent of length of follow-up and outcomes, we projected that among the 219 patients with specimens available, there would be approximately 170×219/563=66 events. Power was then calculated based on a dichotomization of the hsCRP levels (>3.0 mg/L vs. <=3.0 mg/L). Assuming 30% of patients would have an elevated C-Reactive protein level, which was supported by existing literature, we determined that we would have 80% power to detect a hazard ratio (HR) of 2.0 between the high and low CRP groups, based on a one-sided test at the α=0.05 significance level. For overall survival, a total of 102 deaths had been observed, leading to a projected 40 deaths (102*219/563) and 80% power to detect a HR of 2.4. We recognized that power would be low for the distant metastasis endpoint (i.e., distant metastasis at *any* time) given that only 36 events were observed and 26 projected.

Primary Objective and Statistical Methods

Primary analyses compared time-to-event outcomes between patients with low (< or = 3.0 mg/l) vs. high (>3.0 mg/l) CRP levels. The cutpoint, 3.0 mg/l, was based on the observed distribution of hsCRP values in the RTOG 0521 data (approximate 70th percentile) as well as preliminary data supporting this as being an elevated CRP level.[15] The cutpoint of 3.0 mg/l was also considered elevated by the FDA approved clinical assay that was used for this test (Wisconsin Diagnostic Labs, Test Code 1000745, CPT code 86141). Additional analyses treated CRP as a continuous variable. Due to high skewness of the distribution, CRP values were log-transformed.

Probabilities bDFS and overall survival (OS) were calculated using the Kaplan-Meier[18] estimator; the log-rank test was performed for comparisons between the high and low CRP groups. Probabilities of distant metastatic disease were calculated using cumulative incidence curves with death as a competing risk. Both unadjusted and analyses adjusting for treatment arm were performed using Cox[19] proportional hazards regression for bDFS and OS and Fine-Gray[20] regression modelling for distant metastasis. In the case of distant metastases, there were too few events to conduct multivariable analyses and therefore only unadjusted results are presented.

Secondary Objectives

We determined whether each cytokine was associated with the three clinical outcomes (bDFS, distant metastasis, and overall survival) using Cox and Fine-Gray regression as described above. The adjusted estimates for these models included treatment arm as well as (log) CRP, in order to determine whether the cytokine offered added predictive value beyond that associated with CRP. With the exception of MCP, the distributions of the cytokines were strongly positively skewed and therefore a log-transform was first applied. Hazard ratios for these biomarkers are presented per one log increase in the marker. For MCP, the HR corresponds to a one standard deviation (SD) increase in the marker.

Tertiary Objective

Univariate and multivariable logistic regression models were fit to determine whether CRP and the 15 cytokines were associated with each of the five selected adverse toxicity events. These events were cystitis, diarrhea, erectile dysfunction, pollakiuria, and proctitis, all of which were felt to be potentially attributable to RT.

Multiplicity Adjustment:

To determine the significance of the associations adjusting for multiplicity (i.e., to control the family-wise error rate while testing a large number of hypotheses) we applied the minP method as previously described by Westfall and Young[21] and others[22]. This procedure incorporates the correlation among the predictors and is therefore less conservative than a Bonferroni adjustment. Specifically, we held the set of covariates fixed for each patient. We then randomly permuted the observed survival times (and censoring indicator) across the N subjects, thereby simulating a situation in which none of the variables is associated with the outcome. For each permuted dataset, the minimum p-value (two-sided) among the multiple tests of association was obtained. This was repeated R=10,000 times, thereby providing the distribution of the minimum p-value under the null hypothesis. The observed p-value for the actual data was then compared with the upper percentile of this generated distribution.

Results

Patient characteristics

Demographic and clinical characteristics of the patients are shown in Table 1. These patient characteristics are very similar and representative to those of the parent study which has been previously published.[18] A total of 202 patients had valid CRP levels and the table is therefore based on these patients. The CRP values and hsCRP values were similar to

prior publications of CRP values in populations not known to harbor prostate cancer.[23] The median age was 66 years (range 48–83). Approximately 90% of the patients were non-hispanic white and 10% African-American; there were 7 Hispanic/Latino patients. Over 91% of the patients were Zubrod performance status 0 at entry. One hundred-seven patients received AS+RT and 95 AS+RT+CT.

Biochemical Disease-free Survival

The five-year bDFS rate was approximately 80% in both arms. A total of 66 patients had a failure event, which equaled the projected number from the original power calculation. The hazard ratios for CRP dichotomized and for log hsCRP were small and non-significant; therefore we were unable to validate the findings previously reported.[15,16] For each of the biomarkers, two Cox regression models were fit as described above. The first included only the biomarker in question (unadjusted), while the second included the biomarker, treatment arm, and covariates based on the backward elimination procedure described in Materials and Methods (covariate-adjusted). Results are shown in Table 2. Statistically significant associations found were for log IL-6 (p=0.033 unadjusted; p=0.039 adjusted) and log IL-10 (p=0.0030 unadjusted; p=0.0027 adjusted). To adjust for multiplicity, a randomization test was performed including the 18 predictors evaluated in Table 2. The 5th percentile of the minimum p-value was 0.0030934 and therefore the unadjusted finding for IL-10 can be regarded as statistically significant. The randomization test was then re-run on the 17 remaining predictors. Here the 5th percentile of the minimum p-value distribution was 0.0033520 and therefore the result for IL-6 does not reach statistical significance after adjusting for multiplicity. The findings for IL-10 remained statistically significant when adjusting for multiplicity.

Overall Survival and Distant Metastasis:

The five-year survival rates were 86% and 90% in the AS+RT and AS+RT+CT arms, respectively. A total of 62 patients in the cohort died. There were no statistically significant associations between any of the measured cytokine levels and overall survival (data not shown). For distant metastasis, due to the small number of events (36), only unadjusted analyses were run. The results are summarized in Table 3. IL-10 was associated with a higher risk of distant metastatic disease on unadjusted analysis (p= 0.028) with increasing levels of the biomarker conferring increased risk of distant failure However, based on a randomization test, this finding is not statistically significant (5th percentile of the minimum p-value distribution was 0.0032814).

Toxicity

Exploratory associations between the selected inflammatory biomarkers and toxicity were analyzed by fitting unadjusted and adjusted logistic regression models. Pretreatment levels of IFN-γ, IL-1b, IL-2, IL-13, IL-23 were negatively associated with g 2 or higher pollakiuria (adjusted OR=0.64, 0.65, 0.71, 0.72, and 0.74, respectively, all p<0.05) and IL-6 was negatively associated with g 2 or higher ED (OR=0.62, p=0.027). The results are summarized in Supplemental Table 1 *(1SA-1SE)* for those biomarkers in which a significant finding was seen. Other biomarkers assessed demonstrated no significant association with toxicity metrics.

Discussion:

Understanding the interactions between the immune system and response to different oncologic therapies represents an exciting frontier of translational cancer research. RT-induced cell kill has considerable intersection and overlap with the host immune state[1]. Despite this, there remains relatively little research into the pre-treatment host immune state and subsequent responses to RT. We have conducted an examination of the influence of the pre-treatment host immune inflammatory state on clinical outcomes following treatment with radiotherapy and chemotherapy in patients with non-metastatic, high-risk prostate cancer. To conduct this analysis, we used a prospectively acquired serum biospecimen repository collected during a phase III clinical trial, RTOG 0521, the results of which have been previously reported.[18] This study was an *a priori* designed and powered analysis for CRP validation, using previously published retrospective data demonstrating correlations between the immune-inflammatory state and biochemical failure following treatment with RT for prostate cancer[15,16]. The analysis also included unplanned exploratory endpoints with multiple additional cytokines for the intention of generating additional, hypothesis generating, findings.

The primary objective of this analysis was to examine the serum biomarker CRP in a prospectively acquired data set to validate previously reported findings from a retrospective cohort.[15] Specifically, we sought to determine if CRP levels were associated with shorter biochemical disease free survival, as previously published in retrospective cohorts.[15,16] This analysis did not validate our originally hypothesized association between pre-treatment CRP levels and shorter bDFS. We can confidently assert that CRP is indeed not associated with biochemical disease free survival in this population. This validation study highlights the importance of prospectively acquired data sets to validate retrospectively ascertained biomarkers.

In addition to CRP levels, we also collected an array of serum biomarkers, many of which mechanistically overlap with the CRP inflammatory cascade. This was an exploratory analysis, intended to examine for findings beyond CRP, yet mechanistically overlapping. Some statistically positive findings emerged from this exploratory analysis. While interesting, these findings should be interpreted with caution due to the multiplicity of markers evaluated and the fact that the study was not designed to conclusively validate these secondary biomarkers. While we did conduct a multiplicity adjustment strategy, it should also be noted there are indeed multiple published methods of multiplicity adjustment, some of which may show these exploratory findings to be non-significant. Nevertheless, the nominally statistically significant results, using our current methodology presented, should be considered as “hypothesis-generating findings” that present opportunities for future study.

One of the significant exploratory findings was for IL-10, which was significantly associated with bDFS and distant metastasis on unadjusted analysis. The IL-10 finding for bDFS held in the presence of a multiplicity adjustment, however the association with distant metastasis did not. IL-10 represents an anti-inflammatory cytokine, mainly produced by macrophages and T-helper type 2 (Th2) cells.[21] The cytokine IL-10 is well studied in a variety of both benign and malignant conditions.[22,23] With regard to prostate cancer,

there have been several recent publications examining IL-10. Specifically, IL-10 has been examined *in vitro* in prostate cancer cell lines, and it has been shown to interfere with tumor-induced angiogenesis.[24] In addition, IL-10 has recently been shown to induce expression of neuroendocrine markers and PDL1 in prostate cancer cells, raising an interesting hypothesis that IL-10 may promote tumor cell survival through suppression of anti-tumor immunity.[24,25] Important to note is that this is purely a hypothesis generating finding regarding IL-10 that needs additional prospective validation.

Notwithstanding limitations, this finding is the first, to our knowledge, in a prospectively collected cohort of high-risk prostate cancer patients to demonstrate an association with IL-10 and prostate cancer specific outcomes. Such a finding presents a novel opportunity for additional investigations to validate this finding in an independent data set. The finding presented, if confirmed, could represent an important option for unique prognostic and therapeutic strategies in the treatment of high-risk prostate cancer. Specific therapeutic implications could include blocking the effects of IL-10. Such strategies have been shown to potentially improve the immunogenicity of dendritic cells in the presentation of antigenic epitopes to T-cells. Specifically, silencing of endogenous IL-10 in human dendritic cells has been shown to lead to the generation of an improved cytotoxic T-lymphocte (CTL) response.[25] Interestingly, IL-10 has also been shown to activate the STAT5 transcription factor, and blockade of this protein has also been previously demonstrated to sensitize prostate cancer to treatment with RT.[26] Such a pathway presented a potential mechanistic explanation that needs future examination. Exploration into the role of IL-10 in the progression and development of metastasis of prostate cancer is needed, particularly in the context of patients treated with RT.

In addition to prostate cancer-specific oncologic outcomes, we examined toxicity events associated with pre-treatment cytokine levels. The findings are potentially important for future exploration into the intersection of immune response and subsequent radiation induced toxicity. If pre-treatment serum cytokine profiles could be shown to be robustly associated with toxicity events, modulating such pathways may serve as a patient-specific strategy to reduce radiation toxicity. Again, these findings should be viewed entirely as hypothesis generating. Pollakiuria, or frequent daytime urination, was a commonly seen toxicity event in the parent study, RTOG 0521. Pollakiuria was shown to be negatively associated with pre-treatment IFN-γ, Il-1b, IL-2, IL-13, IL-23 levels. IL-6 was negatively associated with grade 2 or higher erectile dysfunction. These data may reflect that certain pre-treatment cytokines portend a worse ability to respond to radiation-induced damage. Despite these significant associations, these findings should again be viewed as entirely preliminary and exploratory. Each of these findings require exploration and validation in similar patient cohorts.

There are limitations to this study that are worth careful consideration. This study had samples selected based on patient participation in the secondary biomarker study of 0521. Given that all patients did not consent for the biomarker component of the parent trial, this introduces the potential for bias. Furthermore, this study only addresses the high-risk patient population and does not address all prostate cancer risk groups. The 0521 population was different than the original retrospective population in which the CRP correlations were

observed[15]. Specifically the 0521 population had more aggressive cancers and higher risk disease overall, this may have presented limitations to this biomarker sub-study. Moreover, there was a risk in this analysis for obtaining false positives given the number of cytokines examined. To mitigate such risk, we therefore conducted a randomization test. This test is less conservative that a Bonferroni adjustment, as it took into account the correlation among the cytokines. Based on this test, the association seen between IL-10 and bDFS remains unlikely to be due to chance. Nonetheless, this association, and several of the other findings demonstrated, are considered exploratory and hypothesis generating, which we denote in the interpretation of these results.

Limitations aside, the study presented carries strengths that warrant discussion. This was a prospectively acquired biomarker data set from a randomized phase III trial. The study was designed *a prior*i and the number of events needed to validate CRP was met. Such a data set is unique, and provides high quality clinical-translational data, with robust and consistent follow up, from which accurate correlations and associations can be drawn. Furthermore, this study presents a relatively novel area of research in prostate cancer, specifically the influence of the pre-treatment host immune state on robustly and consistently measured clinical outcomes in a prospective trial. Research hypothesis and findings presented in this paper are important for radiation oncologists and translational oncology researchers to consider for future research efforts focused on the intersection of the immune state and response to RT. Robust identification of immune biomarkers could enable personalized medical interventions focused on optimizing a specific patient's immune state before starting treatment with RT.

In conclusion, we present the results of a validation study examining the intersection of the pre-treatment host immune state and response to RT. Despite compelling preliminary data, CRP was not associated with poorer prostate cancer-specific outcomes following treatment with RT. Several pre-treatment immune cytokine serum levels were found to be significantly associated with both prostate cancer-specific outcomes and toxicity events. The results of these novel cytokine biomarker analyses should be considered hypothesis-generating and present an opportunity for further exploration and validation. The intersection of the pre-treatment host immune state offers an exciting area of cancer research, ripe for future hypothesis-driven translational work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Sharing Statement:

All data used in the publication will be de-identified and available for data sharing via NCI's NCTN/NCORP Data Archive at least 6 months from the publication date. Data dictionaries are provided with the data. Information about the archive and how to access the data can be found here: https://nctn-data-archive.nci.nih.gov/

References:

1. Stone HB, Peters LJ, Milas L. Effect of host immune capability on radiocurability and subsequent transplantability of a murine fibrosarcoma. J Natl Cancer Inst. Nov 1979;63(5):1229–35. [PubMed: 291749]
2. Sfanos KS, De Marzo AM. Prostate cancer and inflammation: the evidence. Histopathology. Jan 2012;60(1):199–215. doi:10.1111/j.1365-2559.2011.04033.x [PubMed: 22212087]
3. Sfanos KS, Yegnasubramanian S, Nelson WG, De Marzo AM. The inflammatory microenvironment and microbiome in prostate cancer development. Nature reviews Urology. Jan 2018;15(1):11–24. doi:10.1038/nrurol.2017.167 [PubMed: 29089606]
4. de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nature reviews Cancer. Jan 2006;6(1):24–37. doi:10.1038/nrc1782 [PubMed: 16397525]
5. Showalter A, Limaye A, Oyer JL, et al. Cytokines in immunogenic cell death: Applications for cancer immunotherapy. Cytokine. Sep 2017;97:123–132. doi:10.1016/j.cyto.2017.05.024 [PubMed: 28648866]
6. Briukhovetska D, Dörr J, Endres S, Libby P, Dinarello CA, Kobold S. Interleukins in cancer: from biology to therapy. Nature Reviews Cancer. 2021/08/01 2021;21(8):481–499. doi:10.1038/s41568-021-00363-z [PubMed: 34083781]
7. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. The Journal of clinical investigation. Jun 2003;111(12):1805–12. doi:10.1172/JCI18921 [PubMed: 12813013]
8. Hefler LA, Concin N, Hofstetter G, et al. Serum C-reactive protein as independent prognostic variable in patients with ovarian cancer. Clin Cancer Res. Feb 1 2008;14(3):710–4. doi:10.1158/1078-0432.CCR-07-1044 [PubMed: 18245530]
9. Legouffe E, Rodriguez C, Picot MC, et al. C-reactive protein serum level is a valuable and simple prognostic marker in non Hodgkin's lymphoma. Leukemia & lymphoma. Oct 1998;31(3–4):351–7. doi:10.3109/10428199809059228 [PubMed: 9869199]
10. Beer TM, Lalani AS, Lee S, et al. C-reactive protein as a prognostic marker for men with androgen-independent prostate cancer: results from the ASCENT trial. Cancer. Jun 2008;112(11):2377–83. doi:10.1002/cncr.23461 [PubMed: 18428198]
11. O'Brian D, Prunty M, Hill A, Shoag J. The Role of C-Reactive Protein in Kidney, Bladder, and Prostate Cancers. Front Immunol. 2021;12:721989. doi:10.3389/fimmu.2021.721989 [PubMed: 34512646]
12. Schmid M, Schneitter A, Hinterberger S, Seeber J, Reinthaller A, Hefler L. Association of elevated C-reactive protein levels with an impaired prognosis in patients with surgically treated endometrial cancer. Obstetrics and gynecology. Dec 2007;110(6):1231–6. doi:10.1097/01.AOG.0000292085.50987.f2 [PubMed: 18055714]
13. Tartour E, Dorval T, Mosseri V, et al. Serum interleukin 6 and C-reactive protein levels correlate with resistance to IL-2 therapy and poor survival in melanoma patients. Br J Cancer. May 1994;69(5):911–3. [PubMed: 8180022]
14. Tatokoro M, Saito K, Iimura Y, Fujii Y, Kawakami S, Kihara K. Prognostic impact of postoperative C-reactive protein level in patients with metastatic renal cell carcinoma undergoing cytoreductive nephrectomy. The Journal of urology. Aug 2008;180(2):515–9. doi:10.1016/j.juro.2008.04.025 [PubMed: 18550105]
15. ***

16. Thurner EM, Krenn-Pilko S, Langsenlehner U, et al. The elevated C-reactive protein level is associated with poor prognosis in prostate cancer patients treated with radiotherapy. European journal of cancer. Mar 2015;51(5):610–9. doi:10.1016/j.ejca.2015.01.002 [PubMed: 25618827]
17. Hu JJ, Urbanic JJ, Case LD, et al. Association Between Inflammatory Biomarker C-Reactive Protein and Radiotherapy-Induced Early Adverse Skin Reactions in a Multiracial/Ethnic Breast Cancer Population. J Clin Oncol. Aug 20 2018;36(24):2473–2482. doi:10.1200/jco.2017.77.1790 [PubMed: 29989859]
18. Rosenthal SA, Hu C, Sartor O, et al. Effect of Chemotherapy With Docetaxel With Androgen Suppression and Radiotherapy for Localized High-Risk Prostate Cancer: The Randomized Phase III NRG Oncology RTOG 0521 Trial. J Clin Oncol. May 10 2019;37(14):1159–1168. doi:10.1200/JCO.18.02158 [PubMed: 30860948]
19. Wisconsin Diagnostic Labs.
20. Eve Technologies.
21. Westfall PHaY, S. S (Vol. 279). Resampling-based multiple testing: Examples and methods for p-value adjustment. vol 279. John Wiley & Son; 1993.
22. Kennedy PE, Cade BS. Randomization tests for multiple regression. Communications in Statistics - Simulation and Computation. 1996/01/01 1996;25(4):923–936. doi:10.1080/03610919608813350
23. Danesh J, Wheeler JG, Hirschfield GM, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. The New England journal of medicine. Apr 1 2004;350(14):1387–97. doi:10.1056/NEJMoa032804 [PubMed: 15070788]
24. Samiea A, Yoon JSJ, Ong CJ, Zoubeidi A, Chamberlain TC, Mui AL. Interleukin-10 Induces Expression of Neuroendocrine Markers and PDL1 in Prostate Cancer Cells. Prostate Cancer. 2020;2020:5305306. doi:10.1155/2020/5305306 [PubMed: 32802517]
25. Culig Z. Response to Androgens and Androgen Receptor Antagonists in the Presence of Cytokines in Prostate Cancer. Cancers (Basel). Jun 12 2021;13(12)doi:10.3390/cancers13122944
26. Maranto C, Udhane V, Hoang DT, et al. STAT5A/B Blockade Sensitizes Prostate Cancer to Radiation through Inhibition of RAD51 and DNA Repair. Clin Cancer Res. Apr 15 2018;24(8):1917–1931. doi:10.1158/1078-0432.CCR-17-2768 [PubMed: 29483142]

Table 1

Demographics for All Eligible Patients with hsCRP Data

	AS+RT (n=107)	AS+RT+CT (n=95)	Total (n=202)
Age (years)			
Median	68	64	66
Min - Max	48 – 83	49 – 83	48 – 83
Q1 - Q3	61 – 73	60 – 70	61 – 72
< 65	38 (35.5%)	49 (51.6%)	87 (43.1%)
≥ 65	69 (64.5%)	46 (48.4%)	115 (56.9%)
Race			
Asian	0 (0.0%)	1 (1.1%)	1 (0.5%)
Black or African American	9 (8.4%)	11 (11.6%)	20 (9.9%)
White	98 (91.6%)	83 (87.4%)	181 (89.6%)
Ethnicity			
Hispanic or Latino	3 (2.8%)	4 (4.2%)	7 (3.5%)
Not Hispanic or Latino	102 (95.3%)	83 (87.4%)	185 (91.6%)
Unknown	2 (1.9%)	8 (8.4%)	10 (5.0%)
Zubrod Performance Status			
0	100 (93.5%)	84 (88.4%)	184 (91.1%)
1	7 (6.5%)	11 (11.6%)	18 (8.9%)
Baseline PSA			
Median (Min-Max)	17 (2.4–142.6)	16.4 (0.7–120)	16.7 (0.7–142.6)
Gleason			
7	16 (15.0%)	14 (14.7%)	30 (14.9%)
8	32 (29.9%)	27 (28.4%)	59 (29.2%)
9	52 (48.6%)	43 (45.3%)	95 (47.0%)
10	7 (6.5%)	11 (11.6%)	18 (8.9%)
T-Stage			
T1	15 (14.0%)	22 (23.2%)	37 (18.3%)
T2	65 (60.7%)	49 (51.6%)	114 (56.4%)
T3	26 (24.3%)	23 (24.2%)	49 (24.3%)
T4	1 (0.9%)	1 (1.1%)	2 (1.0%)
CRP (mg/dL)			
Mean	0.3	0.5	0.4
Std. Dev.	0.4	1.2	0.9
Median	0.2	0.2	0.2
Min - Max	0.0 – 3.0	0.0 – 11.2	0.0 – 11.2

	AS+RT (n=107)	AS+RT+CT (n=95)	Total (n=202)
Q1 - Q3	0.1 – 0.4	0.1 – 0.4	0.1 – 0.4
hsCRP (mg/L)			
Mean	3.6	4.8	4.2
Std. Dev.	5.5	14.1	10.4
Median	2.0	2.6	2.3
Min - Max	0.2 – 42.1	0.2 – 137.1	0.2 – 137.1
Q1 - Q3	1.1 – 4.1	1.2 – 4.3	1.1 – 4.2

AS- Androgen suppression, RT- Radiation therapy, CT- Chemotherapy, CRP- C-Reactive Protein hsCRP- high sensitivity C-Reactive Protein, Q1-first quartile, Q3- third quartile

Table 2:

Effects of Biomarkers on bDFS

		Unadjusted Model				Covariate-adjusted Model[a]		
Variable	**n/e**	**HR**	**95% CI**	**p-value**	**n/e**	**HR**	**95% CI**	**p-value**
Log hsCRP	202/66	1.07	(0.84, 1.38)	0.58	202/66	1.07	(0.83, 1.38)	0.60
hsCRP dichotomized	201/65	0.95	(0.57, 1.57)	0.83	201/65	0.98	(0.59, 1.61)	0.92
Log CRP	202/66	1.05	(0.83, 1.33)	0.69	202/66	1.05	(0.83, 1.33)	0.70
Log IFN-γ	188/63	1.15	(0.87, 1.54)	0.33	188/63	1.15	(0.86, 1.55)	0.34
Log IL-1B	188/63	1.33	(0.99, 1.80)	0.063	188/63	1.33	(0.98, 1.79)	0.068
Log IL-2	188/63	1.20	(0.98, 1.46)	0.076	188/63	1.20	(0.98, 1.46)	0.08
Log IL-4	188/63	0.98	(0.77, 1.25)	0.89	188/63	0.98	(0.77, 1.25)	0.87
Log IL-5	187/63	1.24	(0.91, 1.69)	0.17	187/63	1.24	(0.91, 1.69)	0.17
Log IL-6	188/63	1.48	(1.03, 2.12)	**0.033**	188/63	1.50	(1.02, 2.19)	**0.039**
Log IL-8	188/63	1.02	(0.69, 1.52)	0.92	183/63	1.01	(0.68, 1.51)	0.94
Log IL-10	188/63	1.60	(1.17, 2.17)	**0.0030**	188/63	1.61	(1.18, 2.20)	**0.0027**
Log IL-12	188/63	1.28	(0.97, 1.69)	0.079	188/63	1.28	(0.97, 1.69)	0.080
Log IL-13	188/63	1.11	(0.94, 1.31)	0.20	188/63	1.12	(0.95, 1.32)	0.19
Log IL-17A	188/63	1.11	(0.87, 1.42)	0.40	188/63	1.11	(0.87, 1.42)	0.41
Log IL-23	188/63	1.11	(0.91, 1.35)	0.31	188/63	1.11	(0.91, 1.36)	0.30
MCP	187/63	1.02[b]	(0.81, 1.29)	0.86	187/63	1.02[a]	(0.80, 1.28)	0.89
Log TNF-α	188/63	0.96	(0.90, 1.03)	0.31	183/63	0.96	(0.89, 1.03)	0.25
Log GM-CSF	188/63	1.07	(0.91, 1.27)	0.40	188/63	1.07	(0.91, 1.26)	0.42

n/e: number of patients/number of events

[a]Models for log(hsCRP), hsCRP dichotomized, and log(CRP) adjusted for treatment arm only; all others adjusted for treatment arm and log(CRP)

[b]per one standard deviation (SD) change, SD=288

Table 3:

Effects of Biomarkers on Time to DM

Unadjusted Model				
Variable	**n/e**	**SHR**	**95% CI**	**p-value**
Log hsCRP	202/36	0.96	(0.72, 1.29)	0.80
hsCRP dichotomized	201/36	1.04	(0.53, 2.04)	0.90
Log CRP	202/36	1.00	(0.75, 1.33)	0.99
Log IFN-γ	188/35	1.06	(0.64, 1.74)	0.82
Log IL-1B	188/35	1.32	(0.88, 2.00)	0.18
Log IL-2	188/35	1.22	(0.93, 1.59)	0.15
Log IL-4	188/35	1.16	(0.81, 1.66)	0.42
Log IL-5	187/35	1.42	(0.99, 2.03)	0.058
Log IL-6	188/35	1.54	(0.96, 2.46)	0.071
Log IL-8	188/35	1.13	(0.74, 1.72)	0.58
Log IL-10	188/35	1.55	(1.05, 2.30)	**0.028**
Log IL-12	188/35	1.06	(0.77, 1.46)	0.73
Log IL-13	188/35	1.16	(0.91, 1.47)	0.22
Log IL-17A	188/35	1.06	(0.74, 1.52)	0.74
Log IL-23	188/35	1.16	(0.84, 1.61	0.37
MCP	187/35	0.97[a]	(0.73, 1.28)	0.83
Log TNF-α	188/35	0.93	(0.85, 1.03)	0.15
Log GM-CSF	188/35	1.12	(0.90, 1.40)	0.30

n/e: number of patients/number of events

[a] per one standard deviation (SD) change, SD=288