A Pilot Study of Rituximab Neoadjuvant Therapy in Patients with High Risk Prostate Cancer Scheduled to Undergo Radical Prostactecomy

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A Pilot Study of Rituximab Neoadjuvant Therapy in Patients with High Risk Prostate Cancer Scheduled to Undergo Radical Prostatectomy

PRINCIPAL INVESTIGATOR
Stephen B. Howell, M.D.
University of California, San Diego
Moores Cancer Center
3855 Health Sciences Drive #0819
La Jolla, CA 92093-0819
Phone: (858) 822-1112
Fax: (858) 822-1111
Email: showell@ucsd.edu

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LIST OF INVESTIGATORS

PRINCIPAL INVESTIGATOR

Stephen B. Howell, M.D.
Department of Medicine
University of California, San Diego
Moores Cancer Center
3855 Health Sciences Drive #0819
La Jolla, CA 92093-0819
Phone: (858) 822-1112
Fax: (858) 822-1111
Email: showell@ucsd.edu

BIOSTATISTICIAN

Karen Messer, Ph.D.
Department of Family & Preventative Medicine
University of California, San Diego
Moores UCSD Cancer Center
3855 Health Sciences Drive #0901
La Jolla CA, 92093-0901
Phone: 858-822-6823
E-mail: kmesser@ucsd.edu

SUB-INVESTIGATORS

Christopher Kane, M.D.
Department of Surgery
University of California, San Diego
Moores Cancer Center
3855 Health Sciences Drive #0987
La Jolla, CA 92093-0987
Phone: (858) 822-6187
E-mail: ckane@ucsd.edu

Christina Jamieson, Ph.D.
Department of Surgery
University of California, San Diego
Moores UCSD Cancer Center
3855 Health Sciences Drive #0987
La Jolla CA, 92093-0987
Phone: (858)-822-2808
E-mail: camjamieson@ucsd.edu

Ahmed Shabaik, M.D.
Department of Pathology
University of California, San Diego
200 W. Arbor Drive #8720
San Diego, CA 92103-8720
Phone: (619) 543-6280
E-mail: ashabaik@ucsd.edu

Michael Karin, Ph.D.
Department of Pharmacology
University of California, San Diego
9500 Gilman Drive #0723
La Jolla, CA 92093
Phone: (858) 534-0872
E-mail: mkarin@ucsd.edu

Frederick Millard, M.D.
Department of Medicine
University of California, San Diego
Moores Cancer Center
3855 Health Sciences Drive #0987
La Jolla, CA 92093-0987
Phone: (858) 822-6185
Email: fmillard@ucsd.edu

James Michael Randall, M.D.
Department of Medicine
University of California, San Diego
Moores Cancer Center
3855 Health Sciences Drive #0987
La Jolla, CA 92093-0987
Phone: (858) 822-5381
Email: jrandall@ucsd.edu

Michael Liss, M.D.
Department of Surgery
University of California, San Diego
Moores Cancer Center
3855 Health Sciences Drive #0987
La Jolla, CA 92093-0987
Phone: (858) 822-7874
Email: mliss@ucsd.edu

Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name: _____

PI Signature: _____

Date: _____

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LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CLL	Chronic Lymphocytic Leukemia
CMP	Comprehensive Metabolic Panel
CRPC	Castration-resistant Prostate Cancer
CTCAE	Common Terminology Criteria for Adverse Events
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
HRPP	Human Research Protections Program
IHC	Immunohistochemistry
IRB	Institutional Review Board
NCI	National Cancer Institute
NHL	Non-Hodgkin's Lymphoma
PSA	Prostate-specific Antigen
SAE	Serious Adverse Event
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
TURP	Transurethral resection of the prostate
ULN	Upper Limit of Normal
WBC	White Blood Cells

STUDY SUMMARY

Title	A Pilot Study of Rituximab Neoadjuvant Therapy in Patients with High Risk Prostate Cancer Scheduled to Undergo Radical Prostatectomy
Short Title	Neoadjuvant rituximab for prostate cancer
Phase	Pilot
Methodology	Open label, non-randomized, single arm
Study Duration	50 days + 3 years of follow-up
Study Center(s)	Single-center: UCSD
Objectives	Primary Objective To determine the histologic response rate in patients with high risk prostate cancer receiving one treatment cycle of neoadjuvant rituximab prior to radical prostatectomy, defined as an extent of B cell infiltration within the tumor region of the prostatectomy specimen of ≤ the 18.2 percentile of the B cell content from 27 historical control samples. Secondary Objectives 1. To determine the effectiveness of neoadjuvant rituximab in the treatment of prostate cancer as evaluated by the serum PSA. 2. To determine the effect of neoadjuvant rituximab on the serum CXCL13 concentration. 3. To determine the effect of neoadjuvant rituximab on peripheral blood B lymphocyte count. 4. To assess the safety and tolerability of neoadjuvant rituximab. 5. To explore the impact of neoadjuvant rituximab on immunohistochemical staining profiles of primary tumors including expression of: IKKα; BMI1; ubiquintinated histone 2A; B cell marker CD20; T cell markers CD3, CD4, and CD8; macrophage markers CD68 and CD11b; αSMA; CXCL13; LTα and LTβ, and LTβR. 6. To understand the long term impact of neoadjuvant rituximab in the treatment of prostate cancer on biochemical relapse based on PSA levels collected during standard of care follow-up.
Number of Subjects	18
Patient Population	Inclusion Criteria 1. Ability to understand and provide written informed consent. 2. Patient has EITHER: • A Kattan nomogram predicted probability of being disease free 5 years after surgery of < 60%, OR • A Gleason sum ≥ 8. 3. Indicated for radical prostatectomy. 4. Eastern Cooperative Oncology Group (ECOG) performance status of 0-1.

	5. Males aged ≥ 18 years. 6. Adequate organ function as defined below measured within 21 days of study entry: Hematology: • Absolute Neutrophil Count (ANC) ≥ 1.5 x 10^9/L • Platelet count ≥ 100 x 10^9/L • Hemoglobin ≥ 9.0 g/dL • White blood cell (WBC) count ≥ 3.0 x 10^9/L Biochemistry: • AST/SGOT and ALT/SGPT ≤ 2 x institution's upper limit of normal (ULN) • Total bilirubin <1.5 times ULN • Serum creatinine and BUN <1.5 times ULN • Na, K Cl, CO_2, Ca, PO_4 within institutional limits Exclusion Criteria 1. Received prior treatment for prostatic adenocarcinoma including prior surgery (excluding TURP), radiation therapy, or chemotherapy. 2. Current or past use of investigational agents within 4 weeks of study enrollment. 3. Use of erectile dysfunction drugs (e.g., Cialis, Viagra) within 14 days prior to treatment or during study. 4. Evidence of metastatic disease on cross sectional imaging or bone scan. 5. History of hepatitis B or C, HIV, tuberculosis or a chronic infection of any type. 6. Positive test results for chronic hepatitis B infection (defined as positive HBsAg serology) 7. Positive test results for hepatitis C (hepatitis C virus [HCV] antibody serology testing).
Study Product(s), Dose, Route, Regimen	Rituximab: 375 mg/m^2 intravenously once weekly.
Duration of administration	One cycle (1 cycle = 28 days)
Reference therapy	Historical response
Statistical Methodology	The null hypothesis is that a single cycle of rituximab will produce a histologic response in 20% or fewer patients. The study will be powered to detect a true response rate of 50% or greater. This trial will use a Simon two stage design with an α of 0.05 and a β of 0.20 (power 0.8). The trial will enroll an initial cohort of 8 patients; if 3 or more have a histologic response the trial will continue and 10 more patients will be entered for a total of 18 patients. If 7 or more patients have a histologic response the null hypothesis will be rejected and it will be concluded that rituximab is associated with histologic response in more than 20% of patients.

1.0 BACKGROUND AND RATIONALE

1.1 PROSTATE CANCER

Prostate cancer is the most frequently diagnosed cancer in men and the second leading cause of cancer-related death in men in the United States. Hormonal ablation, with either surgical or medical castration, is the cornerstone of initial management of advanced prostate cancer. However, almost all patients with distant metastases will develop androgen-independent cancer within 18 to 24 months after castration and succumb to their disease (1, 2). Patients with metastatic castration-resistant prostate cancer (CRPC) have a median survival of 10 to 12 months. Historically, chemotherapy was not considered to have significant activity in metastatic CRPC. However, practice patterns have altered during the last 10 years, in part due to the availability of prostate-specific antigen (PSA) measurements to monitor tumor burden and results of randomized trials. Recent studies have established roles for docetaxel, abiraterone, and the less cross-resistant taxane, cabazitaxel (3). Nevertheless, the prognosis for men with CRPC remains poor and additional approaches to the management of this disease are needed.

1.2 INFLAMMATION AND SIGNIFICANCE IN CANCER

A link between inflammation and cancer was proposed nearly 150 years ago (4). In the past decade, studies using mouse models of human cancer have illustrated several key mechanisms that link inflammation to tumor development and progression (5). Quite frequently, inflammatory signals, mainly cytokines, produced by immune cells within the tumor microenvironment lead to paracrine activation of transcription factors, such as NF-κB and STAT3, within pre-malignant cells, thereby enhancing survival and proliferation of cancer progenitors. Usually, this crosstalk does not involve irreversible genetic changes, although oncogenic mutations can result in up-regulation of chemokine gene expression in malignant cells, thus promoting establishment of a pro-tumorigenic inflammatory microenvironment (6). Nonetheless, chronic inflammation may also enhance tumor initiation and/or malignant progression through induction of oncogenic mutations, chromosomal instability and epigenetic changes (7). The mechanisms and pathways through which inflammation elicits epigenetic changes that contribute to tumor development and progression are unknown.

Inflammation is also of importance in wound repair and tissue regeneration and it has been pointed out that tumors are analogous to wounds that do not heal (8). Furthermore, signaling pathways that play a major role in tissue regeneration and stem cell renewal, namely the Wnt and Hedgehog pathways, are also key players in tumorigenesis (9). However, it is unknown whether and how Wnt and Hedgehog signaling are activated in response to inflammatory signals generated by tissue injury. It is also not clear how tissue injury leads to stem cell activation.

Another signaling pathway involved in inflammation, tissue repair and cancer is the NF-κB pathway (10). Recent studies of the roles of NF-κB transcription factors in cancer have focused on components of the IκB kinase (IKK) complex, namely the IKKα and IKKβ catalytic subunits (11). While both kinases can activate NF-κB-mediated transcription, IKKα also has NF-κB-independent functions in development (12-14) and tumorigenesis (15-17). NF-κB-independent activities of IKKα are particularly evident in prostate cancer. The growth of CRPC depends on emergence of cancer stem-like cells that either do not require androgen signaling for growth and survival, are highly sensitive to castrate levels of androgens such that the low levels present in following castration are still sufficient to support growth (18), or have an alteration in the

androgen receptor that render it constitutively active. Androgen ablation results in an inflammatory response, characterized by lymphocyte infiltration and production of cytokines that activate IKKα in surviving prostate cancer and this, in turn, accelerates the emergence of CRPC (15).

The castration-induced inflammatory response is thought to be triggered by the death of androgen-deprived prostate cancer cells, which causes the release of signals that induce expression of inflammatory chemokines by components of the tumor stroma. This leads to recruitment of a heterogeneous collection of immune cells, of which B lymphocytes are particularly important as they serve as the main source of lymphotoxin, a heterodimeric member of the TNF family that activates IKKα (15). Ablation of B cells or inhibition of their recruitment into the regressing tumors by neutralization of the B cell chemoattractant CXCL13 prevents IKKα activation and delays prostate cancer re-growth. IKKα is not required for primary tumor growth; however, it is required for metastatic spread, and lymphotoxin signaling leads to nuclear accumulation of IKKα (16). However, the molecular mechanisms by which nuclear IKKα enhances the survival and proliferation of progenitor cells that give rise to metastases and prostate cancer are not clear, although it was found that nuclear IKKα promotes metastasis through repression of the *SBP5* gene, which codes for the metastasis inhibitor maspin (16). Previous studies suggested that nuclear IKKα acts as a histone H3 kinase (19, 20), whereas other reports have described an interaction between IKKα and the histone acetylase (HAT) CBP/p300 that results in phosphorylation of the latter (21). IKKα was also reported to regulate expression and activity of transcription factor E2F1 (22) and counteract SMRT repressor activity leading to acetylation of p65/RelA by p300 (23).

1.2.1 Pre-Clinical Studies at UCSD

Recent studies from Dr. Michael Karin's laboratory at UCSD in both a syngeneic and xenograft mouse model of prostate cancer have demonstrated a key role for B cells in the growth of CRPC (15). B and T lymphocyte infiltration was detected in 100% of human prostate cancer samples, but B cells were undetectable in normal prostate or benign prostatic hyperplasia. In the mouse models, castration was found to result in an inflammatory response with infiltration of the tumor with T and B cells. B cell infiltration was in response to increased levels of CXCL13 and resulted in the production of lymphotoxin. Antibody-mediated neutralization of CXCL13 reduced lymphotoxin production.

The re-growth of androgen-independent tumors following castration was found to be dependent on B cells. As shown in Figure 1, reconstitution of host bone marrow with marrow from mice lacking B and T cells (Rag1) that had been supplemented with proficient B cells enhanced the re-growth of androgen-independent tumors, whereas reconstitution with marrow from mice lacking B cells but with added proficient T cells did not. Lymphotoxin expression in regressing tumors was absent unless mice were reconstituted with proficient B cells, and flow cytometry localized lymphotoxin to tumor infiltrating B cells. As shown in Figure 2, treatment of mice with anti-CD20 antibody significantly delayed tumor re-growth (15).

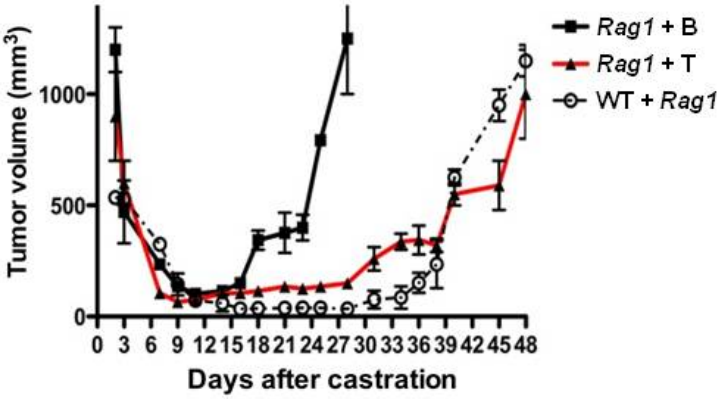


Figure 1. Role of B cells in development of androgen-independent prostate cancer. Myc-CaP tumors were established in WT mice reconstituted with bone marrow from *Rag1*−/− males (n=10) or in *Rag1*−/− males. When tumors reached 1000 mm^3, mice were castrated. Three days before castration, *Rag1*−/− mice (n=10 per group) received via the tail vein purified splenic B or T cells. Tumor volume was measured. Results are averages ± SEM. . Reproduced from reference (15).

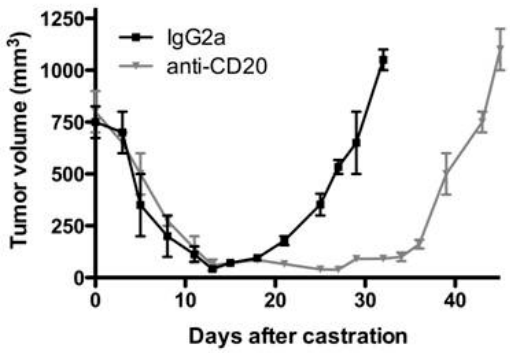


Figure 2. The effect of anti-CD20 antibody treatment on the re-growth of AIPC following castration. *FVB* mice (n=5 each group) were inoculated with myc-CaP cells, castrated as above and were given IgG2a or anti-CD20 (200 µg) every 5 days by IP injection starting 4 days before castration. Tumor volume was measured as above. Results are averages ± SEM. (Karin, M, unpublished).

Prostate cancer allografts from castrated, but not sham-operated, mice exhibited IKKα nuclear translocation. Silencing of IKKα in the tumor cells using siRNA delayed CRPC re-growth. Nuclear translocation of IKKα was dependent on IKKβ in bone marrow B cells. IKKβ deletion abolished lymphotoxin expression by B cells (Figure 3A). To examine whether lymphotoxin production by tumor-infiltrating B lymphocytes stimulates CRPC re-growth, marrow from B-*Ltβ*−/− or T-*Ltβ*−/− mice, which lack lymphotoxin-β, was transplanted into lethally irradiated mice. Lymphotoxin-β ablation in B cells, but not in T cells, delayed growth of CRPC and abolished lymphotoxin-β expression within tumors but did not prevent B cell or macrophage infiltration (Figure 3B). Treatment of mice with the lymphotoxin-βR-Ig decoy was as effective as B cell-specific lymphotoxin-β ablation in delaying CRPC re-growth and prevented IKKα and STAT3 activation (Figure 3C). Silencing of LTβR in Myc-CaP cells also delayed growth of

castration-resistant CaP (Figure 3D). Exogenous lymphotoxin maintained prostate cancer growth in the presence of flutamide in a manner dependent on IKKα whose nuclear translocation was lymphotoxin inducible.

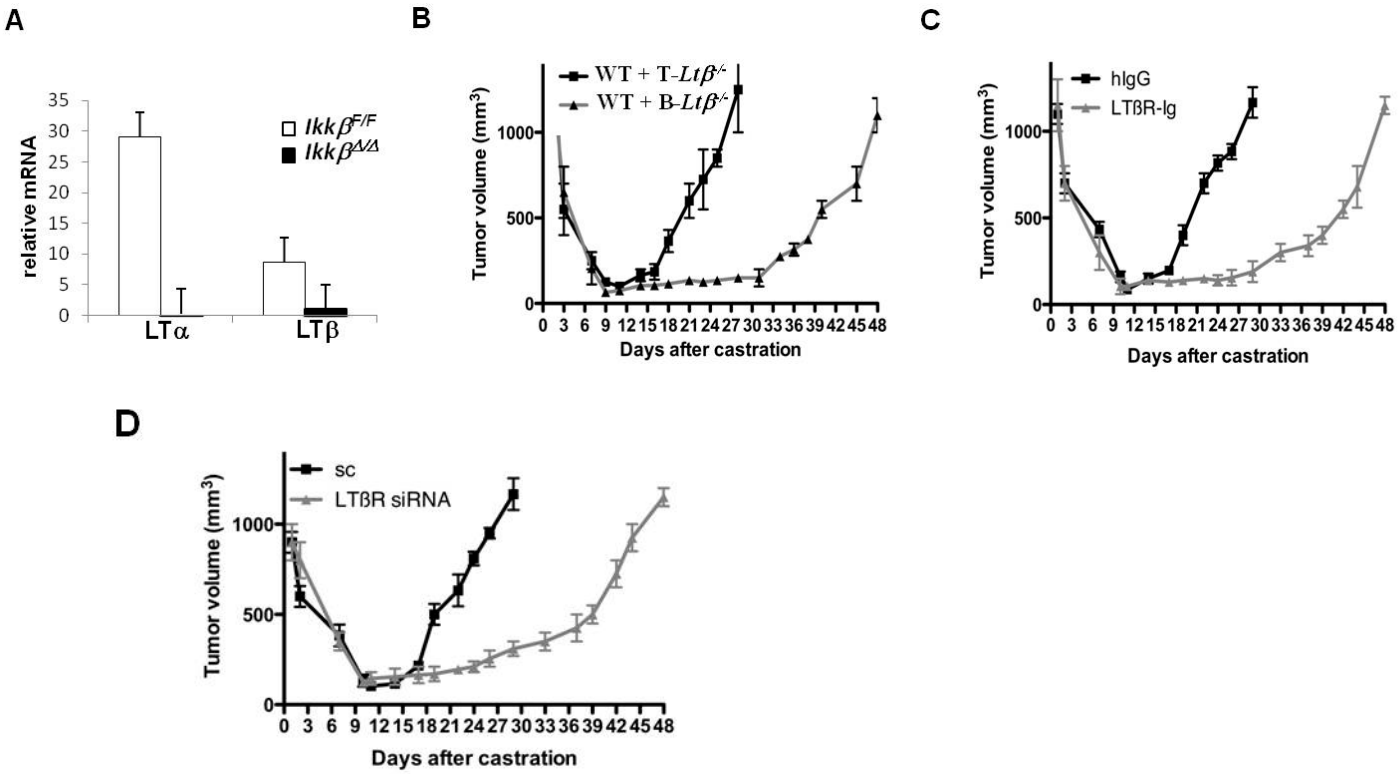


Figure 3. IKKβ-dependent lymphotoxin production by tumor-infiltrating B cells stimulates IKKα-dependent androgen-free survival. ***A.*** RNA from splenic B cells of *Ikkβ*F/F and *Ikkβ*Δ/Δ mice was analyzed for LTα and LTβ expression as above. Results are averages ± SD (n=3). ***B.*** Lethally irradiated *FVB* males were reconstituted with BM from B-*Ltβ*−/− or T-*Ltβ*−/− mice (n=6 per group). After 8 weeks, myc-CaP tumors were established, mice were castrated and tumor volume was measured as above. Results are averages ± SEM. ***C.*** *FVB* mice (n=6 each group) bearing myc-CaP tumors were castrated and given hIgG or LTβR-Ig (100 μg) every 5 days, starting 4 days before castration. Tumor volume was measured as above. Results are averages ± SEM. ***D.*** Tumors were established using myc-CaP cells transduced with lentiviruses expressing scrambled (sc) siRNA or LTβ-specific siRNA. Mice were castrated and tumor volume was measured. Results are averages ± s.e.m. (n=10).

The results from these animal models suggest that an inflammatory response triggered by death of androgen-deprived primary cancer is an important contributor to emergence of CPRC. Tumor infiltrating B cells, which produce lymptoxin-α:β heterotrimers that stimulate the LTβR on prostate cancer cells to induce IKKα nuclear translocation and STAT3 activation, enhance androgen-independent growth. Interference with any component of this response results in a significant and reproducible 3-4 week delay in appearance of CRPC in animal models. Although these inhibitory effects are not absolute, extrapolation from “mouse time” to “human time” suggests that interventions that prevent lymphotoxin production or signaling may delay appearance of CRPC in patients undergoing androgen ablation therapy by 2.3 to 3.1 years. Importantly, the available data suggest that, at least for prostate cancer, the inflammatory

response elicited by the dying primary tumor contributes to the failure rather than the previously proposed success of anti-cancer therapy (24).

1.3 RITUXIMAB

Rituximab (Rituxan®) is a genetically engineered chimeric murine/human monoclonal antibody against the CD20 antigen (human B-lymphocyte-restricted differentiation antigen, Bp35). Initial FDA approval was granted in 1997 for the treatment of patients with relapsed or refractory B-cell Non-Hodgkin's Lymphoma (NHL). In the pivotal study, rituximab caused a rapid depletion of circulating and tissue-based B-cells generally within the first three doses, with sustained depletion for up to 6-9 months after treatment in about 83% of patients. Rituximab has subsequently been approved for several additional indications including chronic lymphocytic leukemia (CLL), rheumatoid arthritis, Wegener's granulomatosis and microscopic polyangiitis. Common adverse reactions observed in clinical trials of lymphoid malignancies were infusion reactions, fever, lymphopenia, neutropenia, chills, infection and asthenia; a more complete listing of adverse reactions are found in Section 3.2.1.

Rituximab is prescribed for NHL patients at 375 mg/m^2 intravenously (IV) once weekly. Four to eight doses are administered, depending on the indication, with a maximum of sixteen doses. For previously untreated and treated CD20-positive CLL, one dose of rituximab 375 mg/m^2 is given prior to fludarabine and cyclophosphamide (FC), followed by 500 mg/m^2 once monthly for 5 months. For the treatment of rheumatoid arthritis 1000 mg rituximab is administered two weeks apart. This study will utilize the schedule approved for the treatment of NHL of 375 mg/m^2 rituximab for 4 weekly doses which should allow for sufficient B cell depletion prior to surgery to detect changes in tumor B cell infiltration.

1.4 RATIONALE

The data from the animal models focused on the emergence of CRPC following castration. Castration induces an inflammatory response that triggers B cell and lymphotoxin-mediated proliferation of androgen-independent cells that can be interrupted with an anti-CD20 antibody. While the optimal time to initiate rituximab therapy may be immediately after the institution of androgen deprivation therapy or cytotoxic chemotherapy due to the high level of inflammation produced by these treatments, it has now been well-documented that essentially all prostate cancers contain inflammatory cells irrespective of their androgen dependency. Since some level of cell death and consequent inflammation is a constant ongoing process in human prostate cancers, treatment with rituximab may be effective when instituted at various stages during the tumor's growth rather than just at the time of peak tumor cell death. While it will eventually be important to determine whether the addition of rituximab to anti-androgen therapy can reduce the emergence of CRPC, there is no medical or ethical basis for doing serial biopsies on patients initiating anti-androgen therapy which precludes the opportunity to study pharmacodynamic endpoints of rituximab treatment. Instead, we will capitalize on the neoadjuvant setting to obtain initial pharmcodynamic evidence of rituximab effect in patients whose original diagnostic biopsy and subsequent radical prostatectomy sample are available for analysis. This is a uniquely appropriate population in which to address the question of whether rituximab can reduce B cell infiltration in prostate cancers and affect histologic markers reflecting B cell drive proliferation.

2.0 STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVES

To determine the histologic response rate in patients with high risk prostate cancer receiving one treatment cycle of neoadjuvant rituximab prior to radical prostatectomy, defined as an extent of B cell infiltration within the tumor region of the prostatectomy specimen of ≤ the 18.2 percentile of the B cell content from 27 historical control samples.

2.2 SECONDARY OBJECTIVES

1. To determine the effectiveness of neoadjuvant rituximab in the treatment of prostate cancer as evaluated by the serum PSA.

2. To determine the effect of neoadjuvant rituximab on the serum CXCL13 concentration.

3. To determine the effect of neoadjuvant rituximab on peripheral blood B lymphocyte count.

4. To assess the safety and tolerability of neoadjuvant rituximab.

5. To explore the impact of neoadjuvant rituximab on immunohistochemical staining profiles of primary tumors including expression of: IKKα; BMI1; ubiquintinated histone 2A; B cell marker CD20; T cell markers CD3, CD4, and CD8; macrophage markers CD68 and CD11b; αSMA; CXCL13; LTα and LTβ, and LTβR.

6. To understand the long term impact of neoadjuvant rituximab in the treatment of prostate cancer on biochemical relapse based on PSA levels collected during standard of care follow-up.

2.3 ENDPOINTS

Primary Endpoint: Histologic response rate after one cycle (28 days) of rituximab. Histologic response is defined as an extent of B cell infiltration within the tumor region of the prostatectomy specimen of ≤ the 18.2 percentile of the B cell content from 27 historical control samples.

Secondary Endpoints:

- Change in PSA from Day 1 to Day 29.
- Change in peripheral blood B cell number from Day 1 to Day 29.
- Change in serum CXCL13 level from screening or Day 1 (prior to drug administration) to Day 29.
- Adverse events (description, timing, grade [CTCAE v4.03], severity, seriousness, and relatedness).
- Change in staining of markers of cell proliferation (Ki67) and apoptosis (TUNEL) between the radical prostatectomy specimen and historical control prostate cancer samples.
- Staining of prostate cancer tissue for IKKα, BMI1, ubiquitinated histone 2A, B cell marker: CD20, T cell markers: CD3, CD4, and CD8, macrophage markers: CD68 and

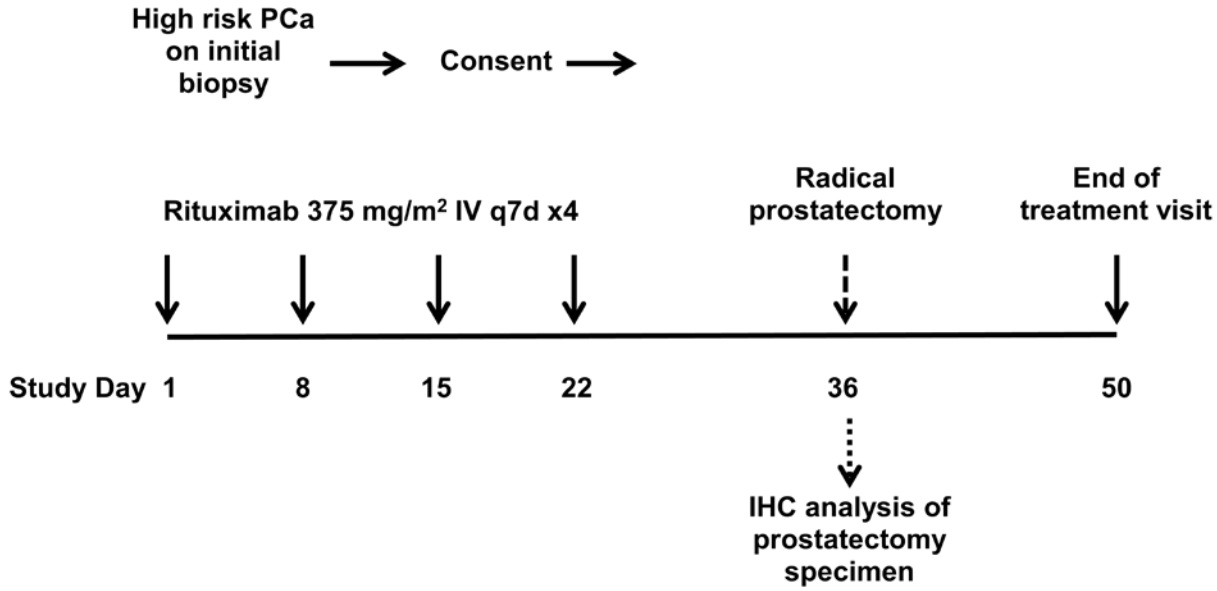
CD11b, αSMA, CXCL13, RANK-L, LTα, LTβ, and LTβ receptor in radical prostatectomy specimen and historical control prostate cancer samples.

- Time to biochemical progression, defined as a single PSA measurement of > 0.2 ng/mL, two PSA values of 0.2 ng/mL, or secondary treatment for increased PSA.

3.0 STUDY DESIGN

3.1 DESCRIPTION OF THE STUDY

This is an open label, single institution study of rituximab neoadjuvant therapy in patients with high risk prostate cancer scheduled to undergo radical prostatectomy. Prior to prostatectomy, patients will receive one treatment cycle (28 days) of rituximab 375 mg/m^2 intravenously once weekly. Patients will be scheduled to undergo radical prostatectomy within two weeks of completing study treatment. Tissue from prostatectomy will be used for immunohistochemistry (IHC) staining of pharmacodynamic markers.



Standard-of-care follow-up visits: measurement of PSA every 3 months for two years and then every 6 months to 3 years

The null hypothesis is that a single cycle of rituximab will produce a histologic response in 20% or fewer patients. The study will be powered to detect a true response rate of 50% or greater. This trial will use a Simon two stage design with an α of 0.05 and a β of 0.20 (power 0.8). The trial will enroll an initial cohort of 8 patients; if 3 or more have a histologic response the trial will continue and 10 more patients will be entered for a total of 18 patients. If 7 or more patients have a histologic response the null hypothesis will be rejected and it will be concluded that rituximab is associated with histologic response in more than 20% of patients. Accrual is expected to occur at the rate of 1.5 patients per month over 12 months.

Patients who are unable to undergo prostatectomy by 28 days after the last dose of rituximab (day 50), whose tumor tissue from radical prostatectomy is not evaluable, or who do not receive all 4 weekly doses of rituximab for reasons unrelated to study treatment or to disease

progression will continue to be followed for safety and for secondary endpoints, including biochemical progression, but will be replaced for the analysis of the primary endpoint.

Patients will also be monitored for an additional 3 years as part of their standard-of-care follow-up visits to determine time to biochemical relapse. Standard-of-care follow-up will include the measurement of PSA approximately every 3 months for the first two years, and then each 6 months for the 3rd year. Biochemical relapse is defined as a single PSA measurement of >0.2 ng/mL, two PSA values of 0.2 ng/mL, or secondary treatment for increased PSA (25).

3.2 SAFETY PLAN

3.2.1 Adverse Events Using Rituximab

Please refer to the package insert for more complete information.

Adverse reactions of any grade have been observed in 99% of patients receiving rituximab for lymphoid malignancies. Severe and life-threatening (grade 3 and 4) events were reported in 57% of these patients.

Infusion reactions, which are sometimes severe and/or fatal, are associated with rituximab. Severe reactions typically occurred during the first infusion with time to onset of 30–120 minutes. Rituximab-induced infusion reactions and sequelae include urticaria, hypotension, angioedema, hypoxia, bronchospasm, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, cardiogenic shock, anaphylactoid events, or death.

General adverse events have been reported with rituximab, although it should be noted that fever, chills, rigors, flushing, and cytokine release syndrome have been associated with infusion-related reactions that can be severe and/or fatal. In clinical trials, general adverse events reported include fever (53%; 5 - 56% combination trials), chills (33%; 3 -13% combination trials), pain (12%), flushing (5%; 14% combination trials), night sweats (15%), peripheral edema (8 -16%), rigors (10% in combination trials), chest tightness (7% in combination trials), and fatigue (13%; 39% combination trials).

Respiratory adverse events have been reported with rituximab, although it should be noted that hypoxia, bronchospasm, pulmonary infiltrates, acute respiratory distress syndrome (ARDS), sneezing, cough, and throat irritation have been associated with infusion-related reactions that can be severe and/or fatal. In clinical trials, adverse events reported include throat irritation (9%; 2% combination trials), increased cough (13%; 15% combination trials), bronchospasm (8%), dyspnea (7-10%), epistaxis (11%), and rhinitis (12%; 3% combination trials).

Cardiovascular adverse events have been reported with rituximab, although it should be noted that hypotension, hypertension, myocardial infarction, ventricular fibrillation, and cardiogenic shock have been associated with infusion-related reactions that can be severe and/or fatal. Hypotension was reported in 10% (2% in combination trials) of patients and hypertension was noted in 6-12% (8% in combination trials) of patients during clinical trials.

Tumor Lysis Syndrome: Acute renal failure, hyperkalemia, hypocalcemia, hyperuricemia, or hyperphosphatemia from tumor lysis, some fatal, can occur within 12–24 hours after the first infusion of Rituxan in patients with NHL. A high number of circulating malignant cells (≥25,000/mm^3) or high tumor burden confers a greater risk of TLS.

Hematologic adverse events have been reported with rituximab therapy. Lymphopenia (48%; 40% Grade 3/4) is the most common hematologic reaction. Other hematologic adverse reactions include leukopenia (10-14%; 4% Grade 3/4), neutropenia (14%; 6% Grade 3/4), thrombocytopenia (12%; 2% Grade 3/4), and anemia (8 -16%; 3% Grade 3/4). In combination trials, cytopenias that had a higher incidence in therapy that included rituximab as compare to the comparator arms included neutropenia (4 -49%), anemia (35%), leukopenia (23%), pancytopenia (3%), thrombocytopenia (9 -11%), and febrile neutropenia (9 -15%). A single case of transient aplastic anemia (pure red cell aplasia) and 2 cases of hemolytic anemia were reported in patients with lymphoid malignancies. Cases of bone marrow aplasia, prolonged pancytopenia, late-onset neutropenia (defined as occurring 40 days after the last dose of rituximab), and hyperviscosity syndrome in patients with Waldenstrom's macroglobulinemia have been reported in post-marketing surveillance.

Hepatitis B virus (HBV) reactivation, in some cases resulting in fulminant hepatitis, hepatic failure, and death **has occurred in patients treated with drugs classified as CD20-directed cytolytic antibodies, including rituximab. Cases have been reported in patients who are hepatitis B surface antigen (HBsAg) positive and also in patients who are HBsAg negative but are hepatitis B core antibody (anti-HBc) positive. Reactivation also has occurred in patients who appear to have resolved hepatitis B infection (i.e., HBsAg negative, anti-HBc positive and hepatitis B surface antibody [anti-HBs] positive). HBV reactivation is defined as an abrupt increase in HBV replication manifesting as a rapid increase in serum HBV DNA level or detection of HBsAg in a person who was previously HBsAg negative and anti-HBc positive. Reactivation of HBV replication is often followed by hepatitis.**

Rituximab-induced B-cell depletion is associated with decreased serum immunoglobulins in a minority of patients. **Serious infections** have been reported, including fatal bacterial, fungal, and new or reactivated viral infections (CMV, herpes simplex virus, parvovirus B19, varicella zoster virus, West Nile virus, hepatitis B and C virus). Onset can occur during therapy and up to one year after completion of rituximab-based treatment. In non-Hodgkin's lymphoma patients, the overall incidence of infection was 31%; 19% of patients had bacterial infections, 10% had viral infections, 1% had fungal infections, and 6% were unknown infections. Serious infection including sepsis has occurred in < 5% of these patients. In rheumatoid arthritis patients 39% of patients and 62% of Wegener's granulomatosis and microscopic polyangiitis patients experienced an infection of any type. Specific infections reported in clinical trials include nasopharyngitis/pharyngitis, upper respiratory tract infections, urinary tract infections, bronchitis, sinusitis (6%), pneumonia, lower respiratory tract infection, cellulitis, sepsis, and colitis. Infectious adverse event reported in post-marketing surveillance include an increase in fatal infections in HIV-associated lymphoma and an increased incidence of Grade 3/4 infections in patients with previously treated lymphoma without known HIV.

Patients with hematologic malignancies or with autoimmune diseases who receive rituximab may develop potentially fatal **progressive multifocal leukoencephalopathy (PML)**. Information suggests that patients with rheumatoid arthritis who receive rituximab have an increased risk of PML. PML is a white matter disease and is characterized by the onset of neurological signs and symptoms including weakness, sensory deficit, hemianopsia, cognitive dysfunction, aphasia, coordination or gait difficulties, and seizures, which progress over time. Mucocutaneous reactions, some with fatal outcomes have been reported with rituximab. These reports include bullous rash/vesicular rash, lichenoid dermatitis (lichen planus-like eruption),

paraneoplastic pemphigus (an uncommon disorder associated with the underlying malignancy), Stevens-Johnson syndrome, and toxic epidermal necrolysis.

Abdominal pain, GI obstruction, and GI perforation, in some cases leading to death, were observed in patients receiving rituximab in combination with chemotherapy. Overall, abdominal pain has been reported in 14% of patients with lymphoid malignancies treated with rituximab and in 2% of rheumatoid arthritis patients treated with rituximab plus methotrexate. The incidence of abdominal pain was high in patients with bulky disease (i.e., a single lesion $\geq$ 10 cm) than those with lesions < 10 cm in diameter.

Nervous system or psychiatric events have been reported with rituximab, although it should be noted that headache, dizziness, and tremor have been associated with infusion-related reactions that can be severe and/or fatal. In clinical trials, adverse events reported include headache (17-19%), dizziness (10%), anxiety (5%; 2% combination trials), peripheral neuropathy (30% combination trials), paresthesias (2% combination trials), migraine (2% combination trials), and insomnia (14%). In post-marketing reports, Posterior Reversible Encephalopathy Syndrome (PRES) and Reversible Posterior Leukoencephalopathy Syndrome (RPLS) have been reported.

Immune/autoimmune events have been reported with rituximab during post-marketing surveillance and include uveitis, optic neuritis, systemic vasculitis, pleuritis, lupus-like symptoms, serum sickness, polyarticular arthritis, and vasculitis with rash.

As with all therapeutic proteins, such as rituximab, there is potential for **immunogenicity**. One percent (4/356) of non-Hodgkin's lymphoma patients tested positive for human anti-chimeric antibody formation (HACA) and 3 patients had an objective clinical response. A total of 11% (273/2578) of rheumatoid arthritis patients tested positive for HACA any time after receiving rituximab and 23% (23/99) patients with Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) tested positive for HACA by 18 months.

Skin/soft tissue or hypersensitivity adverse events have been reported with rituximab, although it should be noted that urticaria, angioedema, anaphylactoid reactions/anaphylactic shock, pruritus, rash (unspecified) have been associated with infusion-related reactions that can be severe and/or fatal. In clinical trials, adverse events reported include rash (10-15%; 17% combination trials), pruritus (14%; 5-17% combination trials), urticaria (8%; 2% combination trials), and angioedema (11%).

Gastrointestinal adverse events have been reported with rituximab, although it should be noted that nausea and vomiting have been associated with infusion-related reactions that can be severe and/or fatal. In clinical trials, adverse events reported include nausea (18-23%; 8% combination trials), vomiting (10%), diarrhea (10-17%), dyspepsia (3% combination trials), and weight gain (11% combination trials).

Musculoskeletal adverse events have been reported with rituximab, although it should be noted that myalgia has been associated with infusion-related reactions that can be severe and/or fatal. In clinical trials, adverse events reported include asthenia (26%; 2% combination trials), myalgia (10%), arthralgia (10-13%; 6-12% combination trials), and muscle spasms (17%).

Metabolic adverse events reported with rituximab therapy include hyperglycemia (9%) and increased LDH (7%).

3.2.2 General Plan to Manage Safety Concerns

A number of measures will be taken to ensure the safety of patients participating in this trial, addressed through exclusion criteria and routine monitoring. Patients will be evaluated for adverse events at each study visit for the duration of their participation in the study and for 28 days after the discontinuation of rituximab.

At least 30 minutes before each rituximab infusion patients will be pre-medicated with diphenhydramine 25 mg intravenously (or another antihistamine) and acetaminophen 650 mg orally. The infusion rate start at 50 mg/h and be advanced progressively (see section 4.2.2) to reduce the risk of infusion reaction. Patients will be monitored closely during each study drug infusion for detection of symptoms or signs of infusion reactions and the infusion rate reduced or stopped based on changes in vital signs and patient appearance and response to treatment. Pre-medications and study drug administration have been specified to help minimize toxicity and guidelines are provided in Sections 4.2.2 and 4.2.3 for study drug modifications.

Positive results of hepatitis B or C screen tests will be included in the patient's electronic medical record at UCSD and will be brought to the attention of the primary UCSD physician for subsequent management.

4.0 MATERIALS AND METHODS

4.1 PATIENTS

4.1.1 Inclusion Criteria

Subjects must meet all of the inclusion criteria to participate in this study.

1. Ability to understand and provide written informed consent.

2. Patient has EITHER:
   - A Kattan nomogram predicted probability of being disease free 5 years after surgery of < 60%, OR
   - A Gleason sum ≥ 8.

3. Indicated for radical prostatectomy.

   Note: candidates for radical prostatectomy are still eligible even if they have a history of deep venous thrombosis, pulmonary embolism, and/or cerebrovascular accident or currently requiring systemic anticoagulation.

4. Eastern Cooperative Oncology Group (ECOG) performance status of 0-1 (*Appendix A*).

5. Males aged ≥ 18 years.

6. Adequate organ function as defined below measured within 21 days of study entry:

Hematology:

- Absolute Neutrophil Count (ANC) $\geq 1.5 \times 10^9$/L
- Platelet count ≥ 100×10^9/L
- Hemoglobin ≥ 9.0 g/dL
- White blood cell (WBC) count ≥ 3.0×10^9/L

Biochemistry:

- AST/SGOT and ALT/SGPT ≤ 2 x institution's upper limit of normal (ULN)
- Total bilirubin <1.5 times ULN
- Serum creatinine and BUN <1.5 times ULN
- Na, K Cl, CO_2, Ca, PO_4 within institutional limits

4.1.2 Exclusion Criteria

Subjects meeting any of the exclusion criteria at baseline will be excluded from study participation.

1. Received prior treatment for prostatic adenocarcinoma including prior surgery (excluding TURP), radiation therapy, or chemotherapy.

2. Current or past use of investigational agents within 4 weeks of study enrollment.

3. Use of erectile dysfunction drugs (e.g., Cialis, Viagra) within 14 days prior to treatment or during study.

4. Evidence of metastatic disease on cross sectional imaging or bone scan.

5. Receipt of a live vaccine within 4 weeks prior to rituximab administration.

6. History of hepatitis B or C, HIV, tuberculosis or a chronic infection of any type.

7. Positive test results for chronic hepatitis B infection (defined as positive HBsAg serology).

8. Positive test results for hepatitis C (hepatitis C virus [HCV] antibody serology testing).

4.2 STUDY TREATMENT

4.2.1 Rituximab Formulation

Rituximab for injection is available in sterile, preservative-free single-use vials [100 mg/10 mL and 500 mg/50 mL].

4.2.2 Rituximab Dosage, Administration, and Storage

Rituximab will be prepared for intravenous administration according to the package insert.

Rituximab will be administered intravenously at a dose of 375 mg/m^2 of body surface area once per week for 4 weeks (Days 1, 8, 15, and 22 of a 28-day cycle). The total dose to be infused on each treatment day will be based on the patient's body surface area at the time of screening and will remain the same throughout the study. Patients that miss a dose for reasons unrelated to toxicity are expected to receive the next dose on schedule.

Rituximab should not be administered as an IV push or bolus.

Premedicate before each infusion:

Diphenhydramine 25 mg intravenously (or another antihistamine) and acetaminophen 650 mg orally should be administered at least 30 minutes prior to rituximab infusion.

Since transient hypotension may occur during rituximab infusion, consideration should be given to withholding anti-hypertensive medications 12 hours prior to rituximab infusion.

First Infusion

Initiate infusion at a rate of 50 mg/hr. In the absence of infusion toxicity, increase infusion rate by 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr.

If an infusion reaction occurs, the infusion should be temporarily slowed or interrupted. The infusion can continue at one-half the previous rate upon improvement of symptoms.

Subsequent Infusions
Initiate infusion at a rate of 100 mg/hr. In the absence of infusion toxicity, increase rate by 100 mg/hr increments at 30-minute intervals, to a maximum of 400 mg/hr.
If an infusion reaction occurs, the guidelines for the first infusion should be followed.

Storage
Unopened vials of rituximab are stable until the expiration date indicated on the package when stored at 2°C–8°C (36°F–46°F). Vials should be protected from direct sunlight and should not be frozen or shaken.

When prepared as directed, rituximab solutions for infusion may be stored at 2°C–8°C (36°F–46°F) for 24 hours. Rituximab solutions for infusion have been shown to be stable for an additional 24 hours at room temperature. However, since Rituximab solutions do not contain a preservative, diluted solutions should be stored refrigerated (2°C–8°C). No incompatibilities between Rituximab and polyvinylchloride or polyethylene bags have been observed.

4.2.3 Rituximab Dose Modifications

There will be no rituximab dosage modification in this study.

Dose Discontinuation Guidelines

Toxicity- NCI CTCAE Grade	Dose Modifications
Infusion Reactions	
Less severe allergic reactions (general itching or rash only)	A. Stop infusion. B. Rapidly evaluate signs and symptoms C. Administer PRN medications (e.g., topical therapy for skin reactions, antipyretics, antihistamines). – If symptoms resolve, resume infusion at least one-half the previous rate.
Severe anaphylactic response	A. Stop infusion IMMEDIATELY. B. Rapidly evaluate signs and symptoms. C. Administer emergency drugs (e.g., epinephrine, famotidine, diphenhydromine, hydrocortisone sodium

	succinate). D. Maintain intravenous line with NS or Lactated Ringer's to expand vascular space. E. Place patient in supine position and reassure patient/significant other. F. Monitor vital signs every 2 minutes until stable, then every 5 minutes for 30 minutes, then every 15 minutes as needed. G. Maintain patient's airway, assessing for increasing edema of respiratory passage. (administer oxygen as needed, anticipate need for CPR). Discontinue study treatment.
Hematologic and Non-Hematologic	
Grade ≤ 1	Continue study treatment without dose or schedule modification.
Grade ≥ 2	Discontinue study treatment.

4.3 CONCOMITANT AND EXCLUDED MEDICATIONS

All concomitant medications and blood products, as well as interventions received by patients from the first dose of study drugs until the end of study visit should be recorded.

4.3.1 Supportive Care Guidelines

Patients should receive full supportive care for disease-related symptoms, including hematopoietic growth factors, anti-emetics, transfusion of blood products, fluid and electrolyte replacement, and antibiotics when appropriate.

4.3.2 Excluded Medications and Treatments

Use of anti-neoplastic or anti-tumor agents not part of the study therapy, including chemotherapy, radiation therapy, immunotherapy, and hormonal anticancer therapy, is not permitted while participating in this study. Concurrent use of investigational agents is not permitted. Concurrent use of erectile dysfunction drugs (Cialis, Viagra) is not permitted.

4.4 STUDY ASSESSMENTS

A flowchart of scheduled study assessments is provided in Appendix B.

All patients will be closely monitored for safety and tolerability during the study and the follow-up period.

4.4.1 Definitions of Study Assessments

a. Medical History

Medical/Oncology history should include smoking history, surgeries, clinically significant diseases within the last 5 years, history of malignancy (date of first diagnosis, disease course, and prior therapies), and all medications taken over the 30 days prior to start of

study treatment (including prescriptions, over-the-counter, and herbal/homeopathic remedies).

b. Demographics
Demographics consist of age, gender, race, and ethnicity.

c. Vital Signs
Vital signs will include measurement of pulse rate, systolic and diastolic blood pressure while the patient is in a seated position, and temperature.

d. Physical Measurements
Physical measure will include body height and body weight.

e. Physical Examination
A complete physical examination should include the evaluation of general appearance; evaluation of head, eyes, ears, nose, and throat (HEENT); and cardiovascular, pulmonary, abdominal, musculoskeletal, skin, lymph nodes, neurological, and genitourinary systems.

Changes from baseline abnormalities should be recorded at each subsequent physical examination. New or worsened abnormalities should be recorded as adverse events if appropriate.

f. Performance Status
Performance status will be evaluated according the ECOG criteria listed in Appendix A.

g. Laboratory Assessments
Laboratory assessments may include the following:

- Hematology
  Including hemoglobin, hematocrit, platelet count, WBC count, and percentage or absolute differential count.

- Comprehensive metabolic panel (CMP)
  Including sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, total protein, albumin, AST, ALT, total bilirubin, and alkaline phosphatase.

- Prognostic marker
  Serum PSA.

- Inflammatory marker
  Serum CXCL13. See Section 4.5.1.

- Hepatitis B and C testing
  Hepatitis B testing includes HBsAg, anti-HBc, and anti-HBs.
  Hepatitis C testing includes antibody (HCV antibody).

h. Pain assessment
Patients should be asked to rate their current overall feeling of pain on a scale of 0-10.

i. Tumor Histology Assessment

Assessment of prostate histology for diagnosis and staging will be performed according to standard institutional practices.

4.4.2 Screening and Pretreatment Assessments

Documentation of the informed consent for screening will be maintained in the patient's research chart. Studies or procedures that were performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

The study population for this trial will consist of patients with high risk prostate cancer scheduled to undergo radical prostatectomy. All patients who meet the inclusion and exclusion criteria will be offered enrollment into this study. To confirm patient eligibility for study participation, the following assessments and procedures will be completed during the screening phase.

All screening procedures must be performed within 21 days prior to registration unless otherwise stated. The screening procedures include:

- Written informed consent (up to two months prior to study treatment)
- Review of inclusion and exclusion criteria
- Complete medical/oncology history
- Documentation of concomitant medications
- Demographics
- Complete physical examination
- Vital signs assessment
- ECOG performance status assessment
- Hematology and CMP
- Assessment of PSA and CXCL13 serum levels (note: if collection of blood for CXCL13 is not performed in screening, it may be collected on Day 1)
- Hepatitis B and C testing

4.4.3 Evaluation and Procedures During Treatment

During the treatment period, a window of +/- 1 days will apply to all visits and assessments, unless otherwise specified. Assessments scheduled on the day of study drug administration should be performed prior to study drug administration, unless otherwise noted.

Day 1

- Documentation of concomitant medications and adverse events
- Physical exam and pain assessment*
- Vital signs before and at the end of the rituximab infusion
- Weight and height
- ECOG performance status*
- Hematology*
- CMP*
- Serum PSA*
- CXCL13* assessment
- Rituximab administration

*note: If procedures performed during screening occur within 14 days of the first administration of study drug, these laboratory assessments do not need to be repeated on Day 1.

Day 8

- Documentation of concomitant medications and adverse events
- Rituximab administration
- Vitals signs before and at the end of the rituximab infusion

Day 15

- Documentation of concomitant medications and adverse events
- Hematology
- Serum PSA
- CXCL13 assessment
- Rituximab administration
- Vitals signs before and at the end of the rituximab infusion

Day 22

- Documentation of concomitant medications and adverse events
- Rituximab administration
- Vitals signs before and at the end of the rituximab infusion

Day 29

- Documentation of concomitant medications and adverse events
- Physical exam and pain assessment
- Vital signs and pain assessment
- Weight
- ECOG performance status
- Hematology
- CMP
- Serum PSA
- CXCL13 assessment

4.4.4 Surgery

Patients will undergo radical prostatectomy according to standard institutional practices. Every attempt will be made to schedule surgery within 14 days after the last dose of rituximab (day 36-50). If the surgery occurs >28 days after the last dose of rituximab (>day 50) the patient will be replaced for the primary outcome and histology based outcomes. The patient will be included in the biochemical recurrence analysis.

4.4.5 End of Treatment Visit

The End of Treatment Visit will occur within 28 days of the last dose of study treatment. For patients completing the full cycle of study treatment, this visit will approximately occur on Day 50 and include the following assessments:

- Documentation of concomitant medications and adverse events
- Physical exam, vital signs, and weight
- ECOG performance status

4.4.6 Follow-up

Patients will be monitored for an additional 3 years as part of their standard-of-care follow-up visits to determine biochemical relapse. Standard-of-care follow-up will include the measurement of PSA every 3 months (+/- 1 month) for the first two years, and then each 6 months (+/- 1 month) during the 3rd year. Biochemical relapse is defined as a single PSA measurement of >0.2 ng/mL, two PSA values of 0.2 ng/mL, or secondary treatment for increased PSA. PSA levels will be collected from patients' medical records and will be entered into the study database.

4.5 ASSAY METHODS

4.5.1 Blood collection for research

Blood (approximately 8 ml) will be collected using 10 mL red top non-anti-coagulated tubes without gel and processed the day of collection for recovery of serum. Samples will be collected during screening (or Day 1), Day 15, and Day 29. Samples will be labeled with the subject's de-identified study number and collection date and delivered to the Moores Cancer Center Biorepository for storage until analysis of CXCL13 levels is performed.

4.5.2 Tissue Procurement

Dr. Ahmed Shabaik will be notified of upcoming prostatectomy of study patients. The entirety of the resected prostate tissue will be processed according to standard institutional procedures. Routine histological assessment for tumor staging will be performed and only then will excess tissue be made available for research purposes.

Samples will be labeled with the subject's de-identified study number and collection date.

4.5.3 Immunohistochemistry

Immunohistochemistry of tumor specimens from surgery and existing historical control prostate tissue will be performed in the laboratory of Christina Jamieson, Ph.D., in the UCSD Moores Cancer Center.

Immunohistochemistry will be performed for the following markers:

- IKKα,
- BMI1,
- Ubiquintinated histone 2A,
- B cell marker: CD20,
- T cell markers: CD3, CD4, and CD8,
- Macrophage markers: CD68 and CD11b,
- αSMA,
- CXCL13,
- RANK-L,
- LTα,
- LTβ, and
- LTβ receptor.

4.6 PATIENT DISCONTINUTATION

Patients may discontinue study treatment at any time. Any patient who discontinues treatment will be encouraged to return to the study center to undergo follow-up assessments. The primary reason for discontinuation should be recorded. Reasons for discontinuation of a patient by the investigator include, but are not limited to, the following:

- Voluntary withdrawal from treatment (follow-up permitted),
- Voluntary withdrawal of consent (termination of treatment and follow-up),
- Patient noncompliance,
- Investigator determination that it is not in the patient's best interest to continue participation,
- Lost to follow-up.

4.7 DATA QUALITY ASSURANCE

Data will be collected via notes in the electronic medical record and outside reports (such as those for laboratory results) and entered into the appropriate Case Report Forms (CRFs) in an electronic database. The study investigator will be responsible for sign off on the eCRFs in a timely fashion, in order that the database can be go live in time for the first patient registration. Data will be submitted within 1 month of completion of the appropriate tests. Data will be monitored by the Moores Cancer Center Clinical Trials Office (CTO), within one month of the first patient completing cycle 1 and every 6 months thereafter, according to the Moores Cancer Center standard data monitoring plan.

5.0 STATISTICAL METHODS

The primary goal of this trial is to determine the histologic response rate in patients with high risk prostate cancer receiving one treatment cycle (28 days) of neoadjuvant rituximab prior to radical prostatectomy. Patients will be considered to have a histologic response if the extent of B cell infiltration within the tumor region is ≤ the 18.2percentile of the B cell content from 27 historical control samples. The threshold value of the 18.2 percentile for histologic response was chosen on the basis of analysis of B cell density in the prostatectomy specimens from 27 untreated high-risk control samples, where high risk is defined as Gleason > 8 or PSA > 20 and more than 1 positive core, or intermediate risk and more than 50% positive cores. The threshold was selected so as to be reasonably confident that the untreated population would have a histologic response rate of ≤20% by this definition. This threshold is based on a 70% lower confidence limit on the 20^{th} percentile of the distribution of B cell density in the 27 untreated high-risk control samples. Note that while an unadjusted 20^{th} percentile could have been used, 18.2 percentile was chosen to be more conservative.

Secondary Endpoints include:

- Change in PSA from Day 1 to Day 29.
- Change in peripheral blood B cell number from Day 1 to Day 29.
- Change in serum CXCL13 level from screening or Day 1 (prior to drug administration) to Day 29.
- Adverse events (description, timing, grade [CTCAE v4.03], severity, seriousness, and relatedness).
- Change in staining of markers of cell proliferation (Ki67) and apoptosis (TUNEL)

between the radical prostatectomy specimen and historical control prostate cancer samples.

- Staining of IKKα, BMI1, ubiquitinated histone 2A, B cell marker: CD20, T cell markers: CD3, CD4, and CD8, macrophage markers: CD68 and CD11b, αSMA, CXCL13, RANK-L, LTα, LTβ, and LTβ receptor in radical prostatectomy specimen and historical control prostate cancer samples.
- Time to biochemical progression, defined as a single PSA measurement of > 0.2 ng/mL, two PSA values of 0.2 ng/mL, or secondary treatment for increased PSA.

5.1.1 Study Design and Justification of Sample Size

This is a single arm, non-randomized, open-label study and utilizes a Simon's optimal two-stage design (26). Suppose 20% is the uninteresting level, i.e., if the histologic response rate is ≤ 20%, we will consider the treatment as not promising. We will test the null hypothesis H0: p≤20% against the alternative hypothesis H1: p>20%, where p is the histologic response rate after one treatment cycle of rituximab. The two stage design proposed below will have 80% power to reject the null and conclude that the true response rate is above 20%, if the true response rate is ≥50%, at 5% significance level.

The study design is described in detail as follows:

Stage 1: 8 subjects will be accrued; accrual will be held up until the response results for all the 8 subjects are known. The trial will be terminated at Stage 1 if ≤2 of the 8 subjects has a response; otherwise it continues to Stage 2.

Stage 2: 10 more patients will be accrued. We will reject the therapy if among all the 18 (8+10) subjects, the number of patients who have a defined response is ≤6. If 7 or more patients have a histologic response the null hypothesis will be rejected and it will be concluded that rituximab is associated with histologic response in more than 20% of patients.

Early stopping probability: Under this design, if the null hypothesis is true, the probability of stopping the trial early will be 80%.

The treatment/control status will be blinded to the operator in the response assessment procedure. At both interim and final analysis, the full set of 27 historical controls will be reassessed concurrently with the treated samples. Slides from the pre-existing historical control prostate tissue samples will be used for IHC. Please see the "PROTUX trial-statistical plan for primary endpoint" document for details.

5.1.2 Analysis of the Study Conduct

Enrollment, eligibility violations, protocol deviations and reasons for patient discontinuation from the study will be summarized.

5.1.3 Analysis of Demographic and Baseline Characteristics

Demographic and baseline characteristics, such as age, race, disease stage, ECOG performance status, will be summarized with use of means, standard deviations, medians, and ranges for continuous variables and with use of proportions for categorical variables.

Study drug administration data will be listed and any dose modifications will be noted. Means and standard deviations will be used to summarize the length of time study drug was received.

5.1.4 Primary Analysis

The primary endpoint is the histologic response rate after one treatment cycle (28 days) of rituximab 375 mg/m^2 intravenously once weekly for 4 weeks. The primary analysis is described in section 5.1.1. The histologic response rate and its associated 95% confidence interval will be estimated.

5.1.5 Secondary Analyses

The analysis for PSA changes from day 1 to day 29 will use all patients that received at least one dose of rituximab. The analysis for peripheral blood B cell number changes from day 1 to day 29 and the analysis for serum CXCL13 level changes from screening or day 1 to day 29 will only use patients who received the full 4 doses of rituximab. Appropriate test (Paired T test or nonparametric Wilcoxon test) will be used to test if there are significant changes before and after the rituximab treatment.

Staining of tissue for IKKα, BMI1, ubiquitinated histone 2A, B cell marker: CD20, T cell markers: CD3, CD4, and CD8, macrophage markers: CD68 and CD11b, αSMA, CXCL13, RANK-L, LTα, LTβ, and LTβ receptor will also be compared between the historical control prostate samples and radical prostatectomy specimen, and appropriate two sided tests at 5% significance level (e.g. nonparametric tests if data are not normally distributed) will be performed to see if the changes are significant or not.

Patients will also be monitored for an additional 3 years as part of their standard-of-care follow-up visits to determine biochemical relapse. They will be monitored every 3 months for the first two years, and then each 6 months up to 3 years, as in our preliminary data (Figure 5). All patients who received a least one dose of rituximab will be included in this analysis. We expect the median time to biochemical relapse for this group of high risk patients to be approximately 36 months (Kane, C, unpublished UCSD data, Figure 6). Time to biochemical relapse will be analyzed using a Cox Proportional Hazards model with time to median relapse compared against an expected value of 36 months. At three years of follow-up, with 18 subjects, this study has 71% power using a one sided, exponential MLE test with $\alpha = 10\%$ to detect a doubling of median survival from 36 months to 72 months (27). This assumes uniform accrual over one year, no loss to follow-up, and exponentially distributed time to relapse.

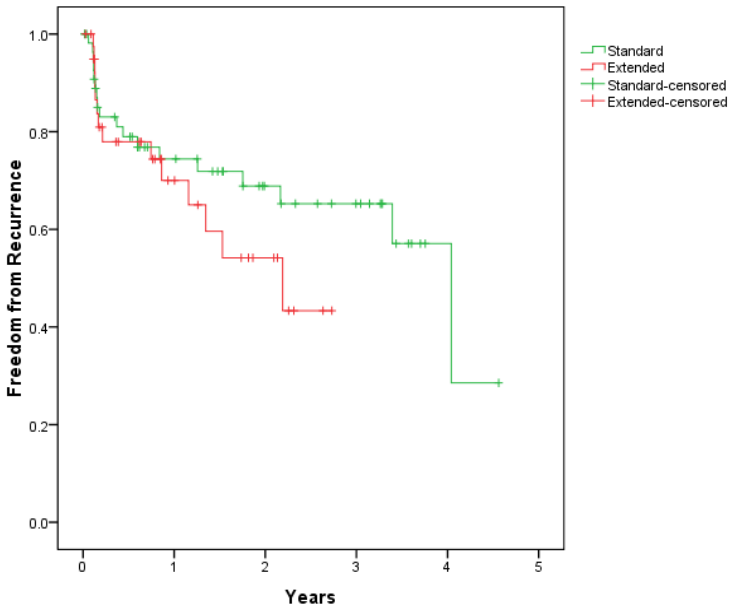


Figure 6. Biochemical relapse following prostatectomy using standard versus extended LND.

Details of any adverse events will be documented. The timing, grade (CTCAE v4.03), seriousness, duration, relatedness to study agent of adverse events will be summarized in a table.

6.0 ASSESSMENT OF SAFETY

The safety of rituximab will be assessed through collection and analyses of adverse events (AEs) and laboratory tests.

6.1 SPECIFICATION OF SAFETY VARIABLES

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs) that are considered related to rituximab, all events of death, and any study specific issue of concern.

6.1.1 Adverse Events

An adverse event (AE) is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational medicinal product (IMP) or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with **Prostate Cancer** that were not present prior to the AE reporting period.
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as cardiac catheterizations).

- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

6.1.2 Serious Adverse Events

An AE should be classified as an SAE if:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the patient at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization.
- It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to the investigational product.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above).

All AEs that do not meet any of the criteria for serious should be regarded as non-serious AEs.

The terms "severe" and "serious" are not synonymous. Severity (or intensity) refers to the grade of a specific AE, e.g., mild (Grade 1), moderate (Grade 2), or severe (Grade 3) myocardial infarction. "Serious" is a regulatory definition (see previous definition) and is based on patient or event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations from the Sponsor to applicable regulatory authorities.

Severity and seriousness should be independently assessed when recording AEs and SAEs on the CRF.
The AE grading (severity) scale found in the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), Version 4.03, will be used for AE reporting. The CTCAE v4.03 is available at http://ctep.cancer.gov/reporting/ctc.html

6.2 METHODS AND TIMING FOR ASSESSING AND RECORDING SAFETY VARIABLES

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, as outlined in Section 5.1.1, are collected and reported to the FDA, UCSD IRB, and Genentech, Inc. in accordance with CFR 312.32 (IND Safety reporting).

6.2.1 Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of any study procedures and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

6.2.2 Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the rituximab (see following guidance), and actions taken.

To ensure consistency of AE and SAE *causality assessments*, investigators should apply the following general guideline:

Yes
There is a plausible temporal relationship between the onset of the AE and administration of rituximab, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to rituximab; and/or the AE abates or resolves upon discontinuation of rituximab or dose reduction and, if applicable, reappears upon re-challenge.

No
Evidence exists that the AE has an etiology other than the rituximab (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to rituximab administration (e.g., cancer diagnosed 2 days after first dose of rituximab).

Expected adverse events are those adverse events that are listed or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

6.2.3 Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation timepoints should be adopted. Examples of non-directive questions include:

"How have you felt since your last clinical visit?"
"Have you had any new or changed health problems since you were last here?"

6.2.4 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

a. Diagnosis vs. Signs and Symptoms
If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently

available. If a diagnosis is subsequently established, it should be reported as follow-up information.

b. Deaths

All deaths that occur during the protocol-specified AE reporting period (see Section 5.1.2), regardless of attribution, will be reported to the appropriate parties. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report “Unexplained Death”.

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

d. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:
Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

e. Pregnancy

If a female subject becomes pregnant while receiving investigational therapy or within 90 days after the last dose of study drug, a report should be completed and expeditiously submitted to the Genentech, Inc. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to the investigational product should be reported as an SAE.

f. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior investigational product exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

g. Reconciliation

h. The Sponsor (principal investigator) agrees to conduct reconciliation for the product. Genentech and the Sponsor will agree to the reconciliation periodicity and format, but agree at minimum to exchange monthly line listings of cases received by the other party. If discrepancies are identified, the Sponsor and Genentech will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

i. **Non-Serious AE Reporting**
All Non-serious Adverse Events originating from the Study will be forwarded, in a quarterly report to Genentech.

6.2.5 MedWatch 3500A Reporting Guidelines

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form (http://www.fda.gov/medwatch/index.html):

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information
Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B., initials, subject number), protocol description and number, if assigned, suspect drug, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report.)

Occasionally, Genentech may contact the reporter for additional information, clarification or current status of the subject for whom an adverse event was reported. For questions regarding STEAE reporting, you may contact the Genentech Drug Safety representative noted below.

MedWatch 3500A form is available at
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

6.3 REPORTING REQUIREMENTS FOR ADVERSE EVENTS

6.3.1 Expedited Reporting of Serious Adverse Events

- The Principal Investigator must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug.

- Investigators must report all SAEs to Genentech within the timelines described below. The completed Medwatch/case report should be faxed immediately upon completion to Genentech Drug Safety at:

  (650) 225-4682
  OR
  (650) 225-5288

  The Safety Reporting Fax Cover Sheet is found in Appendix C.

  Relevant follow-up information should be submitted to Genentech Drug Safety as soon as it becomes available.

  - Serious AE reports that are <u>related</u> to the investigational product will be transmitted to Genentech within fifteen (15) calendar days of the Awareness Date.
  - Serious AE reports that are <u>unrelated</u> to the investigational product will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.
  - Any reports of pregnancy following the start of administration with the investigational product will be transmitted to Genentech within thirty (30) calendar days of the Awareness Date.

- The UCSD Human Research Protections Program (HRPP) must be notified within 10 business days of “any unanticipated problems involving risk to subjects or others” (UPR).

  The following events meet the definition of UPR:

  1. Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
  2. Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
  3. Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
  4. Any new information (e.g., publication, safety monitoring report, updated sponsor safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
  5. Any breach in confidentiality that may involve risk to the subject or others.
  6. Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

- *Post-marketing 15-Day "Alert Report"*

  The Principal Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is **unexpected and assessed by the investigator to be possibly related to the use of Rituximab**. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be submitted to the FDA (2 copies) at the following address: Central Document Room, 12229 Wilkins Avenue, Rockville, MD 20852.

  All Postmarketing 15-Day "Alert Reports" submitted to the FDA by the Sponsor-Investigator must also be faxed to:

  Genentech Drug Safety

  Fax: (650) 225-4682 or (650) 225-4683

  (Please use the Genentech safety reporting fax cover sheet for the fax transmission)

  For questions related to safety reporting, contact:

  Genentech Drug Safety

  Tel: 1-888-835-2555

  Or

  Fax: (650) 225-4682 or (650) 225-4683

  (Please use the Genentech safety reporting fax cover sheet for the fax transmission)

6.3.2 Routine Reporting of Adverse Events

- The UCSD HRPP will be notified of any adverse events that are not unanticipated problems involving risk to subjects or other (non-UPRs) at the time of the annual Continuing Review.

6.4 TYPE AND DURATION OF FOLLOW-UP OF PATIENTS AFTER ADVERSE EVENTS

All AEs and SAEs that are encountered during the protocol-specified AE reporting period should be followed to their resolutions, or until the investigator assesses them as stable, or the patient is lost to follow-up. Resolution of AEs and SAEs (with dates) should be documented on the appropriate AE or SAE CRF page and in the patient's medical record to facilitate source data verification.

For some SAEs, follow-up may occur by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

7.0 INVESTIGATOR REQUIREMENTS

7.1 OBLIGATIONS OF INVESTIGATORS

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

7.2 CONFLICT OF INTEREST

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed according to UCSD conflict of interest policy.

7.3 INSTITUTIONAL REVIEW BOARD (IRB) APPROVAL AND CONSENT

The IRB should approve the consent form and protocol prior to any study-related activities. It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

7.4 SUBJECT DATA PROTECTION

In accordance with the Health Information Portability and Accountability Act (HIPAA), subjects who have provided written informed consent must also sign a subject authorization to release medical information to the study Sponsor and allow a regulatory authority, or Institutional Review Board access to subject's medical information relevant to the study.

7.5 DATA AND SAFETY MONITORING/AUDITING

In addition to adverse event monitoring and clinical oversight by the principal investigator and co-investigators, quality assurance of the study will be performed by the clinical trials office internal monitor. Standard monitoring reports will be generated by the Biostatistics Shared Resource at two weeks after the first patient visit, and every 3 months or 6 months thereafter.

This study will also use the UCSD Moores Cancer Center Data Safety and Monitoring Board (DSMB) to provide oversight in the event that this treatment approach leads to unforeseen toxicities.

Data from this study will be reported to the DSMB after the first stage of the study (after 8 subjects) or annually, whichever comes first. If the study proceeds to the second stage, reports to the DSMB will be made annually.

If data capture is at UCSD, statisticians from the Biostatistics Shared Resource will prepare reports for data monitoring and DSMB review. The report will include following components:

- accrual summary by month and by treatment group (if any);
- study status summary including total number of accrual as of date, numbers of subjects who are on/off study, on study treatment(s), on study but off study treatment(s);
- summary statistics for baseline variables that are crucial to study design and analysis;
- data completeness report that summarizes number of observed data points vs. number of expected data points;
- name, grade, onset date and relation to study treatment of adverse events, including all laboratory tests and hematological abnormalities, signs and symptoms from physical examination findings, and spontaneous reports of adverse events reported to the investigator by participants;
- listings of study treatment (s) and their corresponding dosages, including treatment start date, treatment modification reasons, dates and off treatment date.
- In addition, treatment response will be reported by presenting number of the cases exhibiting a partial response or complete response, number of progressive diseases and deaths.

7.6 STUDY MEDICATION ACCOUNTABILITY

All study drug required for completion of this study will be commercially supplied.

Study drug accountability records will be maintained in accordance with the regulations and institutional practices.

7.7 RECORD RETENTION

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Study documents should be kept on file until three years after the completion and final study report of this investigational study.

8.0 REFERENCES

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9.0 APPENDICES

APPENDIX A. PERFORMANCE STATUS

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework or office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about >50% of waking hours
3	Capable of only limited self-care, confined to a bed or chair >50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

APPENDIX B. SCHEDULE OF EVENTS

Procedure	Screen Day -21 to Day -1	Study Treatment					Surgery	End of Treatment Visit	Follow-Up #
		Day 1	Day 8	Day 15	Day 22	Day 29	Day 36*	Day 50^	
Informed consent	X[g]								
Medical history	X								
Demographics	X								
Eligibility criteria	X								
Concomitant Medications	X	X	X	X	X	X		X	
Adverse Events		X	X	X	X	X		X	
Vital signs [a]	X	X[b]	X[b]	X[b]	X[b]	X		X	
Weight and Height		X				X		X	
Physical Exam	X	X¥				X		X	
ECOG Performance Status	X	X¥				X		X	
Hematology [c]	X	X¥		X		X			
Serum chemistries [d]	X	X¥				X			
Hepatitis B and C testing [e]	X								
PSA assessment	X	X¥		X		X			X
CXCL13 assessment	X	X¥		X		X			
Pain assessment		X				X			
Histology assessment [f]							X		
Rituximab Administration		X	X	X	X				
Prostatectomy							X		

Procedures should be performed on schedule +/- 1 day unless otherwise indicated.

* Radical prostatectomy should be performed as close to day 36 as possible but in any case before day 50.

^ End of treatment visit to occur within 28 days of the last dose of study drug. Patients who discontinue study drug early should be seen within 28 days, which will be earlier than Day 50.

Follow-up consisting of PSA assessment will occur during routine office visits every 3 months (+/- 1 month) for the first two years then every 6 months (+/- 1 month) during the third year.

¥ If hematology, serum chemistries, PSA assessments, physical exam, and ECOG performed during screening occur within 14 days of the first administration of study drug, these procedures do not need to be repeated on Day 1. If research blood collection for CXCL13 assessment occurred during screening, then it need not be repeated on Day 1 (alternatively- if blood not collected during screening then it should be collected on Day 1).

a. Vital signs to include heart rate, blood pressure, and temperature.

b. On infusion days, vital signs to be recorded both before and at the end of rituximab infusion.

c. Hematology: hemoglobin, platelets, total white blood cell count, and differential.

d. Complete Metabolic Panel (CMP) to include: glucose, BUN, creatinine, sodium, potassium, chloride, bicarbonate, calcium, total protein, albumin, AST/SGOT, ALT/SGPT, alkaline phosphatase and total bilirubin.

e. Hepatitis B and C testing: Screen all patients for HBV infection by measuring HBsAg, anti-HBc, and anti-HBs before initiating treatment with rituximab and HCV antibody.

f. Histology assessment: to include routine analysis for tumor diagnosis and staging, plus assessment of pharmacodynamic markers specified in Section 4.5.3 for research.

g. Informed consent may be obtained up to two months prior to study treatment.

APPENDIX C. GENENTECH SAFETY REPORTING FAX COVER SHEET

Genentech
A Member of the Roche Group

SAFETY REPORTING FAX COVER SHEET
GENENTECH SUPPORTED RESEARCH

AE / SAE FAX No: (650) 225-4682

Alternate Fax No: (650) 225-5288

Genentech Study Number	
Principal Investigator	
Site Name	
Reporter name	
Reporter Telephone #	
Reporter Fax #	

Initial Report Date	[DD] / [MON] / [YY]
Follow-up Report Date	[DD] / [MON] / [YY]

Subject Initials (Enter a dash if patient has no middle name)	[ ] - [ ] - [ ]

SAE or Safety Reporting questions, contact Genentech Safety: (888) 835-2555

PLEASE PLACE MEDWATCH REPORT or SAFETY REPORT BEHIND THIS COVER SHEET