Trargeting Her-2 with the Monoclonal Antibody 2C4 in Hormone Refractory Prostate Cancer

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A. Study Purpose and Rationale

Prostate cancer affects about 185,000 men each year, and the incidence is increasing as PSA is being used to diagnose the disease earlier. About 40,000 of' those men die of their prostate cancer each year (Crawford, et al., 1999). As 1020% of cases are metastatic at diagnosis, and approximately 50% of patients eventually fail treatment for localized disease, the incidence of metastatic disease is high. Androgen therapy is the standard of care for metastatic disease, which initially results in tumor regression in the large majority of patients: it causes a decrease in PSA by over 95% in 90-95% of men who are treated. Unfortunately, 98% of patients with metastatic prostate cancer develop progressive disease in 2-5 years after starting androgen therapy. Progression is defined as increase in PSA, worsening symptoms, or disease progression on imaging studies (Small, et al., 1999). These patients are termed "androgen insensitive" or "hormone refractory". I will refer to them as "hormone refractory" from now on. Hormone-refractory prostate cancer (HRPC) is defined as disease progression in the presence of castrate levels of testosterone. As androgen therapy is rarely curative the hormone refractory patients make up a sizable group.

There is no curative therapy available at this time for hormone-refractory prostate cancer. The current standard of care is palliative therapy with mitoxantrone and prednisone (Knox, et al., 2001). This drug combination has been approved by the Food and Drug Administration for the palliative treatment of'HRPC. Tannock et al. (1996) showed decreased pain in 29% of those treated with mit.oxantrone-prednisone compared with 12% receiving prednisone alone. In addition, there was a greater than 50% reduction in PSA in 33% of patients in the mitoxantrone-predrusone arm versus 22% of patients receiving prednisorie alone. There was also improvement in other quality of life measurements, such as relief of constipation and improved mood. However, none of the studies with mitoxantrone-prednisone have shown any survival benefit in HRPC, with most patients in both arms surviving 10-30 months from the beginning of the study (Tannock et al., 1996). A more recent large randomized study of mitoxantrone-hydrocortisone vs. hydrocortisone in HRPC by Kanitoff et al. (1999) showed similar results, with significant improvement in quality of life parameters but no difference in survival (median survival of 12.3 months for the mitoxantrone hydrocortisone and 12.6 for hydrocortisone alone). Therefore, more worK needs to be done to find agents that improve survival in patients with HRPC.

Targeting of the Her-2-neu receptor tyrosine kinase in advanced breast cancer is an important paradigm for therapy directed against a biologic determinant in a solid tumor (Scher, 2000). Herceptin (trastuzumab)., an antibody that targets tile extracellular domain of the HER2 gene product, has been found to be effective in treatment of *Her-2-neu* positive metastatic breast cancer and is FDA approved for - this purpose. In breast cancer, it is theorized that HER-2 activation indirectly leads to phosphorylation and activation of the estrogen receptor in the absence of estrogen (Pletras et al., 1995). Similarly, it has been postulated that in prostate cancer, over expression of HER-2 can activate genes downstream of the androgen receptor in the absence of androgen receptor ligand (Craft et al., 1999, Yeh et al., 1999). In fact, Craft et al. (1999) demonstrated, that overexpression H*er-2-neu* confers androgen independent growth to the androgen-dependent LNCaP prostate cancer cell line. Likewise, overexpression of *Her-2-neu* has been associated with the progression to androgen independence in prostate cancer tissue samples. Signoretti et al. (2000) have demonstrated HER-2 protein overexpression in 25% of untreated primary prostate tumors, 59% of localized tumors after neoadjuvant hormone therapy, and 78% of castrate metastatic tumors. Scher et al. (2000) obtained similar results. In their study, HER-2 overexpression was seen in 20% oflocalized untreated tumors, 68% of localized tumors after androgen deprivation, and 80% or castrate

metastatic lesions in a retrospective analysis, As the castrate metastatic lesions are found in patients with hormone refractorv disease, HER-2 overexpression is the highest in this population. Therefore, HER-2 is a promising target for treatment of these tumors.

Studies of trastuzumab have been done both in human prostate cancer xenograft models (Agus et al., 1999) and in a Phase II clinical trial (Morris et al., 2002) with disappointing results. Agus et al. (1999) found that trastuzumab causes significant growth inhibition in the andiogen-dependent Her-2 expressing human xenograft models in nude mice, but there was no growth inhibition in the androgen- independent Her-2 expressing xerlograft models. Morris et al. (2002) conducted a Phase 11 clinical trial with trastuzumab + paclitaxel in four groups of patients: Her-2 positive androgen-dependent, Her-2 negative androgen-dependent, Her-2 positive androgen- independent, and Her-2 negative androgen-independent. Only the Her-2 negative androgen-independent arm (14 patients) progressed to completion with trastuzumab as a single agent, and there was disease progression seen in all of these patients. Of the 6 Her-2 positive androgen-independent patients who were treated with trastuzumab alone, all progressed by 12 weeks. No larger-scale clinical trials with trastuzumab have been performed yet, but these preliminary results suggest that trastuzumab ryiay not have clinical utility in HRPC. This may be because crosstalk between the Her-2 receptor and activated androgen receptor may be required for the response to trastuzumab in prostate tumors (Agus et al., 2000).

A different anti-Her-2 antibody, the recombinant humanized monoclonal 2C4 antibody (rhumAb 2C4), has been shown to cause growth inhibition in androgenindependent prostate cancer human cell lines and human xenograft mouso models (Agus el. al.,2000; Mendoza et al., 2002). RhumAb 2C4 binds to a surface of the HER2 extracellular domain, preventing the association of HER2 with other receptors of the HER family (Agus et al., 2000). This is an important mechanism because HER-2 functions as a coreceptor with other HER receptors to amplify and/or initiate receptor-ligand signaling. Forexample, association of HER2 with HER3, which is devoid of enzymatic activity, to form the HER2/HER3 receptor complex leads to potent downstream activation of the Ras/MAPK and P13K pathways when activated by ligand. It has been shown that activation of HER2 can lead to induction of androgen target genes; through the MAPK pathway (Yeh et al., 1999). Thus, disruption of the HER2 complexes with other HER receptors is a potential mechanism for growth inhibition in HRPC. Agus et al. (2000) have found that rhumAb 2C4 can inhibit the growth of both androgen-dependent and androgen-independent prostate tumors grown as xenografts in athymic mice. This is in contrast to trastuzumab, which only inhibits grown in androgen-dependent tumors (Agus et al., 1999). Mendoza at al. (2002) have obtained similar results with rhumAh 2C4 on cell proliferation in the androgen- independent prostate cancer cell line 22Rv1 and on tumor growth in a human xenograft model using 22Rv1 cells implanted into nude mice. They also showed that rhumAb 2C4 acts by inhibiting phosphorylation and thereby actilvation of the HER2 receptor. The activity of rhumAb 2C4 has not yet been tested in any clinical trials.

I propose to initiate a Phase III randomized double-blinded clinical trial to study the effects of the recombinant humanized monoclonal 2C4 and Her-2 antibody in patients with honnone refractory prostate cancer when used in conjuction with mitoxantrone and prednisone compared with mitoxantrone/prednisone alone. The primary end point will be survival, while the secondary end points will be quality of life. This study will be undertaken assuming that rhumAb 2C4 has been shown to be safe and at least minimally effective in Phase I and Phase II trials. My primary hypothesis is that rhurnAh 2C4 will improve 12-month survival of patients with HRPC from 50% to at least 70%.

B. Study Design and Statistical Analysis

I will recruit patients with biopsy-proven metastatic adenocarcinema of the prostate who have undergone one prior hormonal manipulation (medical or surgical) and achieved castrate levels of testos-terone (<50 no/ml), but were then found to have disease progression within 2 months of randomization. The study will be advertised to medical oncologists and urologists who treat patients with I-IRPC, and these physicians will be asked to refer appropriate patients for the study. I will define progression as increase in PSA on two consecutive measurements one week apart, a positive bone scan,

and symptoms that include pain. I will select only patients with bone metastases because this is a preferred site for testing Her-2 expression. Of patients with metastatic prostate cancer, 80-90% will have bone metastases (Knox et al., 2,001). Patients who have not had an organiectomy will be required to continue the androgen therapy that they are currently receiving to maintain testosterone of <50 ng/ml.

These patients will be required to have adequate hepatic, renal, cardiac, and bone marrow function. Exclusion criteria will therefore include abnormal creatinine, abnormal liver function tests, ejection fraction <55% on MUGA, granulocyte count <1500/microliter, and platelet count less than 100,000. These exclusion criteria have been chosen based on the known serious toxicities of mitoxanlrone, which can include neutropenia, thrombocytopenia, and congestive heart failure (Tannock et aL, 1996). The toxicities of RhumAb 2C4 are not yel. known in humans, but they will be assumed to be minimal for the putposes of this proposal.

After obtaining informed consent, patients who meet the above eligibility criteria will undergo bone marrow biopsy to obtain tumor tissue from the most easily accessible site of bone metastasis seen on bone scan. This tissue will he used to quantify HER-2 protein expression using immunohistochemistry on biopsy specirnens with the A0485 anti -Her-2-neu antibody (Dako Corp.) at a 1:200 dilution. An absolute scoring system will be used to evaluate Her-2-neu expression: specimens containing >50% Her-2-neu-positive cells will be classified as "positive" (Signorettl et al., 2000). It is important to assess Her-12 expression spocifically at the time of androgen treatment failure rather than at the time of initial diagnosis, as Her-2 expression has been shown to vary between different stages of prostate cancer (Signorettl et al., 2000). Based on the studies of Signorettl et al. (2000) and Scher et al. (2000), 1 would expect about 80% of the patients in my group to have Her-2-neu positive metastatic tumors.

I will seek to enroll 220 Her-2-neu-positive patients who meet the eligibility criteria, who will then be randomized to either the mAb 2C4 + mitoxantrone + prednisone arm or the mitoxantrone + prednisone arm. As determined by Chi square analysis, I will need a minimum of 102 patients in each arin to detect an increase between 50% and 70% survival to 12 months with 80% power. I will need to perform a multi-center trial with approximately four participating i'nedium-size cancer centers to achieve adequate enrollment within a one-year period. IRB approval will be obtained from each institution that participates in the study.

My primary end point will be overall survival. The mean survival in HRPC patients treated with mitoxantrone and steroids is 12.3 months (Kintoff et al., 1999). 1 will use the Chi square analysis to determine any differences between the two arms in the percent of patients surviving to 12 months. I will then construct a Kaplan-Meyer curve to compare survival over time. I will not permit crossover between the two arms, so that any difference in survival will be detectable.

My secondary endpoint will be quality of life. To assess quality of life, I will use the European Organization for Research and Treatment of Cancer QLQ-C30 quality of life questionnaire, which has been designed to quantify quality of life in cancer patients (Aaronson et al., 1993). The QLQ-C30 incorporates nine multi-item scales: five functional scales (physical, role, cognitive, emotional, and social); three symptom scales (fatigue, pain, nausea, and vorniting), and a global health and quality-of-life scale. The score on this 30-item questionnaire can be reported on a scale of 1-100. This questionnaire will be administered to all patients in the study at multiple points in time. At each time point, I will use Chi Square analysis to determine if there is any difference between the two arms in tile percentage of patients who have a 20-point improvement oil this 100-point scale.

C. Study Procedure, Drugs, and Questionnaire

I plan to continue the study for four years, as only about 10% of HRPC patients treated with mitoxantrone/prednisonc survive past 40 months (Tanock et al., 1996; Kantoff et al., 1999). This period of time will allow me to detect significant differences in survival, as well as differences in quality of life between the two study arms.

During patient selection, all prospective enrollees will undergo baseline blood tests, including CBC, basic metabolic panel, hepatic function tests, coagulation panel, testosterone level, and PSA. All candidates will also undergo a bone scan to determine the location of bone metastases. This is a painless radiological procedure that takes less than one hour to perform arid causes no pain and minimal discomfort to the patient. All candidates with positive bone scans will then undergo, at least one bone marrow biopsy. This is a procedure in which bone arid bone marrow is removed using a large bore needle under local anesthesia. This can cause considerable pain and discomfort, but it only takes about 15-20 minutes to perform. There is a possibility that the initial biopsy will not obtaill sufficient turnor cells for analysis, and a repeat bone marrow biopsy may be necessary.

Patients who are enrolled in the study will be examined at 3-week intervals, and blood tests including CBC, basic metabolic panel, hepatic function tests, coagulation panel, testosterone level, and PSA will be repeated at each visit. Patients in the treatment arm will be given 50irng/kg of rhumAb 2C4-subcutaneously twice a week. RhumAb 2C4 is an experimental therapy that has not yet been tested in clinical trials. The above dose was chosen based on studies of RhumAb 2C4 in human xenograft mouse models (Mendoza et al., 2002). 1 am designing this protocol under the assumption that this would be the optimal (lose and route as delineated in a Phase I clinical trial.

All patients will be given oral prednisone 5mg daily. Prednisone is a corticosteroid that has been FDA-approved at this dose by this route for the palliative treatment of HRPC when used in conjuction with mitoxantrone. Prednisone is relatively safe when used at this low dose, but it has been associated with weight gain, immunosuppression, mood changes, psychosis, fluid rctention, leukocytosis, decreased glucose tolerance, and GI bleeding.

All patients will receive mitoxantrone 12mg/m2 body-surface area by IV injection every 3 weeks. Mitoxantrone is an anthracenedione chemotherapeutic agent that has been FDA-approved at this dose by this route for the palliative treatment of 1-HRPC when used in conjuction with prednisone. Known tox1cities of mitoxantrone include neutropenia, thrombocytopenia, congestive heart failure, renal failure, mucositis, and seizures. Chemotherapy will be delayed or adjusted based on observed toxicities. Additional studies will only be performed when clincially indicated based on new symptoms or abnormal blood tests. Prochlorperazine will be used as the anti-emetic medication. Analgesic medications will be adjusted as needed. Patients will be given stool-.softcners as needed. All patients will be administered the QLQ-C30 questionriaine at the time of randomization and again at 6 weeks, 12 weeks, and then at 12 week intervals.

D. References:

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