Clinical Trials of Cancer Therapies Targeting Prostate-Specific Membrane Antigen

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Abstract: Prostate cancer is the most common non-cutaneous cancer of men in the United States and represents their second-leading cause of cancer-related death. Metastatic disease is largely resistant to conventional chemotherapies, and targeted therapies are urgently needed. Prostate-specific membrane antigen (PSMA) is a prototypical cell-surface marker of prostate cancer. PSMA is an integral, non-shed, type 2 membrane protein with abundant and nearly universal expression in prostate carcinoma, but has limited extra-prostatic expression. In addition, PSMA is expressed in the neovasculature of other solid tumors. These findings have spurred development of PSMA-targeted therapies for cancer, and first-generation products have entered clinical testing. Vaccine approaches have included recombinant protein, nucleic acid and cell-based strategies, and anti-PSMA immune responses have been demonstrated in the absence of significant toxicity. Therapy with drug-conjugated and radiolabeled antibodies has yielded objective clinical responses as measured by reductions in serum prostate-specific antigen and/or imageable tumor volume. However, responses were observed in a minor fraction of patients and at doses near the maximum tolerated dose. Overall, these initial studies have provided measured proof of concept for PSMA-based therapies, and second-generation antibody and vaccine products may hold the key to exploit PSMA for molecularly targeted therapy of prostate and other cancers.

Key Words: Prostate-specific membrane antigen, prostate cancer, immunotherapy, antibody-drug conjugate.

INTRODUCTION

Prostate cancer is the most commonly diagnosed non-cutaneous malignancy in American males, with an estimated 234,460 new cases and 27,350 deaths in 2006 [1]. The most potent risk factor for prostate cancer is age, with approximately 80% of cases diagnosed in men over 65 years of age and a median age of 74 for the onset of clinical symptoms [2]. As the population in the United States and other industrialized countries ages, the treatment and management of prostate cancer will increasingly become a major concern.

Approximately 85% of men with prostate cancer present with localized disease, which typically is treated by surgery, radiation or cryotherapy. Androgen deprivation therapy is indicated for individuals with recurrent disease following primary therapy and for individuals who present with metastatic disease. Primary androgen deprivation leads to symptomatic and biochemical improvement in approximately 80% of patients, but the disease eventually becomes refractory to hormone treatment in essentially all patients. Hormone-refractory prostate cancer has proven to be resistant to most conventional chemotherapies, and docetaxel in combination with prednisone currently is the only therapy that has been demonstrated to provide a survival advantage. However, the survival benefit of docetaxel/prednisone therapy is modest (~2.5 months), and new therapies are urgently needed for advanced prostate cancer. Molecular screening techniques have identified a number of proteins associated with prostate cancer. Prostate-specific membrane antigen (PSMA) is one such prostate-restricted protein with important implications for the diagnosis, management and treatment of the disease.

PSMA BIOLOGY

PSMA is the prototypic cell-surface marker of prostate cancer and is abundantly expressed in non-prostatic tumor neovasculature: PSMA was originally defined by the monoclonal antibody (mAb) 7E11 derived from mice immunized with a partially purified membrane preparation from the LNCaP human prostatic adenocarcinoma cell line [3]. 7E11 staining indicated that its antigen was highly restricted to normal and malignant prostate. However, this mAb recognizes an intracellular portion of PSMA that is not accessible on viable cells [4], and the potential of PSMA as a target for antibody and vaccine therapies was not fully recognized until the PSMA gene was cloned and shown to encode a transmembrane protein with a large extracellular domain [5,6].

The PSMA gene consists of 19 exons that span approximately 60 kb of genomic DNA and encodes a type II transmembrane protein with a short, amino-terminal cytoplasmic tail (19 amino acids), a hydrophobic transmembrane domain (24 amino acids), and a large extracellular domain (707 amino acids) at the carboxy-terminus [5,7]. The deduced amino acid sequence contains 10 canonical sites for *N*-linked glycosylation, and carbohydrate moieties are critical for PSMA function and cellular trafficking [8-10].

PSMA is a non-shed, integral membrane protein. The extracellular domain mediates homodimer formation and shares modest homology with the transferrin receptor (TfR) and members of the M28 family of co-catalytic aminopeptidases [5,11,12]. In normal prostate, PSMA exists primarily as a splice variant (designated PSM') that lacks the transmembrane domain [13].

Initial analysis of PSMA expression revealed that PSMA was highly restricted to secretory cells within the prostatic epithelium. Furthermore, PSMA immunoreactivity was absent to moderate in most hyperplastic and benign tissues; however, malignant tissues stained with the greatest intensity [3]. Subsequent studies have confirmed that increased expression of PSMA is nearly a universal feature of prostatic carcinoma (Table **1**) [14-24]. These reports further demonstrated that PSMA expression increases precipitously in a manner directly related to tumor aggressiveness. PSMA expression is highest in high-grade tumors, metastatic lesions and androgen-independent disease (Table **1**). These features make PSMA an excellent target for new therapies.

In addition, several studies have demonstrated that PSMA is abundantly expressed on the new blood vessels that supply most non-prostatic solid tumors, including lung, colon, breast, renal, liver and pancreatic carcinomas as well as sarcomas and melanoma [17-19,26,27]. PSMA is expressed in the endothelial lumen and is accessible to antibody [28]. Importantly, PSMA is not found on normal vasculature [17-19,26,27]. These findings raise the possibility

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Table 1. Overview of Studies that have Assessed PSMA Expression Within Benign Tissues and Tumors of Prostatic Origin

Prostate Tissues	Horoszewicz, 1987 [3]	Lopes, 1990 [24]	Israeli, 1994 [6]	Troyer, 1995 [14]	Wright, 1996 [15]	Silver, 1997 [18]	Liu, 1997 [17]	Kawakami, 1997 [16]	Sweat, 1998 [21]	Bostwick, 1998 [25]	Chang, 1999 [19]	Chang, 2001 [20]	Burger, 2002 [22]	Ross, 2003 [23]
Normal	9/9	2/2	1/1	5/5					100%	184/184	28/28		11/11	
BPH	5/7		1/1	2/4										
PIN	9/9												17/19	
Adenocarcinoma		10/10	1/1	3/4	25/25	33/35	21/21	15/15	100%	129/129	12/12			138/138
Metastases										184/184				
Lymph node	2/2					7/8			98%			6/6		
Bone						8/18						7/7		
Soft tissue												6/6		
Omentum												2/2		
Liver												1/1		

Numerator denotes the number of specimens positive for PSMA expression and the denominator represents the total number of specimens examined. BPH = benign prostatic hyperplasia, PIN = prostatic intraepithelial neoplasia.

that PSMA-targeted therapies may have broad utility for the treatment of other cancers. The role of PSMA in neovascularization has not been established. A recent study in mice has suggested that PSMA interacts with filamin A and impacts β1 integrin signaling in endothelial cells in a pathway involving p21-activated kinase-1 activation [29]. This study is the first to report expression of murine PSMA in mouse neovasculature, suggesting avenues for further research.

Isolation of the PSMA gene facilitated studies into its distribution on normal human tissues. PSMA has limited extraprostatic expression but has been reported in brain, small intestines, liver, proximal kidney tubules and salivary gland [3,6,14,24,30,31]. PSMA expression in prostate cancer is approximately ten-fold greater than that in normal prostate. Expression in normal prostate is approximately 10-fold greater than that in the brain and is 50- to 100-fold greater than that of the liver or kidney. In most tissues, no expression is observed.

PSMA possesses carboxypeptidase activity: PSMA hydrolyzes carboxy-terminal glutamate residues and has been designated human glutamate carboxypeptidase II (GCP II, EC 3.4.17.21). Carboxypeptidase activity is manifested *in vitro* as both folate hydrolase activity and *N*-acetylated α-linked acidic dipeptidase (NAALADase) activity [32,33], and studies have demonstrated that PSMA is identical to enzymes that were previously known as brain NAALADase [31,32] and intestinal folylpoly-γ-glutamate carboxypeptidase [34]. An arginine-rich patch mediates PSMA's substrate specificity [35-37]. Dimerization is critical for enzyme activity [11], and recent crystal structures have revealed that the extracellular domain of PSMA has a compact dimer interface [35-37].

In brain, PSMA hydrolyzes the abundant neuropeptide *N*-acetylaspartylglutamate (NAAG) to liberate *N*-acetylaspartate and glutamate. Glutamate is the primary excitatory neurotransmitter in humans. In preclinical studies, inhibition of PSMA activity has been shown to attenuate stroke, diabetic neuropathy, epilepsy, head trauma, amyotrophic lateral sclerosis (ALS), Alzheimer's disease, Huntington's disease and Parkinson's disease (reviewed in [38]). In the small intestine, PSMA plays a role in the nutritional availability of folate. All animals and plants store folate intracellularly in a poly-γ-glutamated form that is inefficiently absorbed. In the small intestine PSMA removes the γ-linked glutamates, freeing monoglutamyl folate for membrane transport via the reduced folate carrier [33,39]. The roles of PSMA expression in other normal tissues and in malignancy have not been established.

Subcellular trafficking of PSMA: Subcellular localization and intracellular trafficking of a given protein play crucial roles in modulating its physiological function as well as its clinical utility. PSMA is rapidly and constitutively internalized in both prostate and endothelial cells in processes that are at least in part clathrin dependent [40-42]. PSMA's endocytic activity has been linked to an MXXXL motif within its cytoplasmic domain [41]. PSMA internalizes efficiently in the presence of antibody, and this property facilitates the intracellular delivery of cytotoxic drugs and radionuclides [43-49]. On the other hand, internalization of PSMA may affect its utility as a target for unmodified antibodies that rely on recruiting immunologic effector functions.

Polarized epithelial cells have distinct apical and basolateral plasma membranes separated by tight junctions, which can limit access of macromolecules and immune cells to basolateral membranes [50]. In *in vitro* cell culture systems, PSMA is observed on both basolateral and apical surfaces; however, the majority (~70%) is targeted to the apical plasma membrane [8,51]. Depolymerization of microtubules with nocodazole, vincristine or vinblastine redirected PSMA to the basolateral plasma membrane and increased uptake of antibodies [8]. Microtubule disruption affects the localization of syntaxin 3, a t-SNARE necessary for apical targeting of PSMA [52]. Prostate cancer often is characterized by the presence of well-differentiated tumor cells with apical and basolateral membrane domains discernible even at metastatic sites [52], and microtubule disruption with *Vinca* alkaloids or similar agents might have added benefit for PSMA-targeted therapies [52]. Additional studies are needed to explore this issue and to more fully characterize the subcellular localization of PSMA in progressive prostate cancer.

Potential utility of PSMA as a biomarker, prognostic indicator, and therapeutic target for prostate cancer: PSMA's restricted pattern of expression, its upregulation in advanced disease and its membrane-bound nature combine to make this molecule potentially useful for the detection, management and treatment of prostate cancer. ProstaScint™ (Cytogen Corporation, Princeton, NJ) is an ^{111}In-labeled form of mAb 7E11 that has received FDA approval for the immunoscintographic detection and imaging of metastatic prostate cancer in soft tissues [24,53,54]. Because the 7E11 epitope is located in the cytoplasmic domain of PSMA, it is likely that this mAb localizes to regions of tumor necrosis *in vivo*.

As summarized above, immunohistochemical analyses have demonstrated intense upregulation of PSMA in advanced prostate cancer, and PSMA expression was found to independently predict disease outcome through a variety of biological endpoints [23]. Compared to specimens that exhibited only moderate PSMA expression, those with intense expression had significantly higher preoperative levels of prostate-specific antigen (PSA), higher Gleason scores, more advanced tumors, and increased incidence of aneuploid tumors. Elevated levels of PSMA also independently predicted biochemical disease relapse [23]. While all prostate cancers expressed PSMA in this study, patients with higher expression of PSMA pre-prostatectomy were observed to relapse earlier than those with lower expression. The findings were confirmed by a recent study that reported PSMA to be independently associated with the risk of PSA recurrence [55]. Thus, assessment of PSMA levels, either alone or in combination with PSA status, may one day prove useful for the diagnosis of primary or occult metastatic prostate cancer, risk assessment, and the prognosis of disease course.

CLINICAL TRIALS OF MONOCLONAL ANTIBODIES

Radiolabeled huJ591 in prostate cancer: J591 (BZL Biologics, Framingham, MA) is a mouse mAb to the external domain of PSMA. J591 was generated by Dr. Neil Bander and colleagues at the Cornell-Weill School of Medicine and was demonstrated to bind PSMA on nonpermeabilized cells with nanomolar affinity [17,40]. In preclinical studies, radiolabeled forms of murine J591 were shown to specifically target and eliminate PSMA-positive human prostate cancer cell lines *in vitro* and in mouse xenograft models [43,44,47,56].

Based on such findings, murine J591 was humanized or "deimmunized" in a process that involved fusing human IgG1,κ constant regions with J591 variable regions and then removing putative B-cell and T-cell epitopes within the variable regions. Putative B-cell epitopes were removed by replacing surface-exposed framework residues within the J591 variable regions with corresponding germ-line human residues. Potential T-cell epitopes were similarly removed following comparison of J591 framework variable regions with a database of peptides known to bind human major histocompatibility complex class II molecules. Deimmunized J591 (huJ591) was observed to retain favorable properties in terms of its affinity and specificity for PSMA.

Phase 1 trials were performed to assess the safety, dosimetry, and pharmacokinetics (PK) of radiolabeled huJ591 in prostate cancer patients. Radioconjugates were prepared using the β-emitting isotopes ^{90}Y- and ^{177}Lu, and these constructs were evaluated in 2 separate clinical trials [57,58]. Radioconjugates were created by linking radioisotopes to huJ591 via a 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) chelator. Patients enrolled into the trial were those with hormone-refractory prostate cancer with evidence of biochemical progression or radiographic evidence of progression by bone scan, computed axial tomography or magnetic resonance imaging.

The phase 1 trial involving ^{90}Y-labeled mAb enrolled 29 patients [57]. Initially, patients received mAb labeled with the γ-emitting isotope ^{111}In to determine PK, biodistribution and dosimetry. Total-body images were obtained with a gamma camera 1h following administration of the antibody, followed by subsequent imaging on day 1, 2, 3 and either day 6 or 7. Imaging with ^{111}In-labeled mAb demonstrated significant uptake in the liver with lesser uptake in spleen and kidneys. In addition, the mAb specifically targeted sites of metastatic disease in both bone and soft tissue. The half-life for ^{111}In-labeled huJ591 was 32±8 hours. ^{90}Y-labeled huJ591 with a specific activity of 3-5 mCi/mg was administered one week later at one of five initial dose levels: 5, 10, 15, 17.5 or 20 mCi/m^2. Four patients received an initial dose of 5 mCi/m^2, 7 patients received 10 mCi/m^2, 8 patients received 15 mCi/m^2, 6 patients received 17.5 mCi/m^2 and 4 patients received 20 mCi/m^2. Redosing was determined by recovery of platelet and neutrophil counts. One patient in the 15 mCi/m^2 group died of an unrelated pulmonary embolism. Two patients who received an initial dose of 20 mCi/m^2 developed grade 3 thrombocytopenia with bleeding episodes, and these toxicities were considered to be dose limiting. Two patients developed grade 3 anemia, one who received 10 mCi/m^2 and another who received 15 mCi/m^2. Three patients were re-dosed with ^{90}Y-labeled mAb. Two patients received 17.5 mCi/m^2, and one patient received 20 mCi/m^2. Of these 3 patients, one patient who received 17.5 mCi/m^2 and a patient who received 20 mCi/m^2 developed grade 3 thrombocytopenia and neutropenia. Other side effects included fatigue, anorexia, nausea, and elevated levels of transaminases (n=11). One patient developed an upper extremity deep vein thrombosis secondary to a central venous catheter. The maximum tolerated dose was determined to be 17.5 mCi/m^2. No human anti-human antibody responses were observed. Although the study was not designed to assess efficacy, PSA stabilization was noted in 6 patients. In addition, declines in serum PSA of 85% and 70% were observed in 2 patients. PSA levels in these two patients remained decreased for 8-9 months post-treatment.

Another phase 1 trial assessed the safety, dosimetry, and PK of ^{177}Lu-labeled huJ591 [58]. The study accrued 35 patients. Eligibility criteria were similar to those employed for the trial of ^{90}Y-labeled huJ591. Total-body images were obtained with a gamma camera within 1h post-treatment and then on day 2, day 4 or 5, day 6 or 7, and day 12 or 14. Various initial dose levels were investigated, including 10, 15, 30, 45, 60, 70, and 75 mCi/m^2. Retreatment doses were also evaluated. Plasma half-life was 39±13 hours. Of patients receiving 75 mCi/m^2 (n=3), one patient developed grade 4 thrombocytopenia and 2 patients developed grade 3 thrombocytopenia. Also, grade 4 neutropenia occurred in all 3 patients. Of patients receiving 70 mCi/m^2, 2 patients developed grade 4 neutropenia, and one patient developed a dose limiting toxicity of grade 4 thrombocytopenia. Subsequently, 70 mCi/m^2 was determined to be the maximum tolerated dose. Significant hematological toxicity was noted in patients re-dosed with 45 mCi/m^2. However, re-treatment with 30 mCi/m^2 was better tolerated. Within this trial, a PSA decline of ≥ 50% was observed in 4 patients, and PSA stabilization was noted in 16 patients. Of note, responders to ^{177}Lu-huJ591 were patients with elevated PSA levels and without measurable disease at the outset of the study. In contrast, patients who responded to ^{90}Y-huJ591 were those with measurable disease.

Myelosuppression was the most common and severe side effect of treatment with ^{90}Y- and ^{177}Lu-labeled huJ591. Vallabhajosula and colleagues evaluated the myelotoxicity observed in the phase 1 studies [59]. For ^{90}Y-huJ591, there was no clear correlation between myelotoxicity and radioactive dose administered. For ^{177}Lu-huJ591, however, myeltoxicity and especially thrombocytopenia correlated well with both radioactive dose administered and the bone marrow radiation dose. The authors suggested that fractionated doses of ^{177}Lu-huJ591 could be further studied in combination with taxane.

Notably, no significant target-related toxicity was observed in the trials of radiolabeled huJ591. In addition, the majority of bone and soft tissue metastases were targeted with this mAb to the extracellular domain of PSMA. In contrast, ProstaScint™ is less efficient in imaging bone metastases. In certain cases, huJ591 detected not only sites that were observed with technetium scanning, but also sites that were first judged to be false positives on technetium scan and were confirmed to be positive on later scans.

huJ591 in advanced prostate cancer: A pilot study of huJ591 trace-labeled with ^{111}In was conducted in patients with hormone-refractory metastatic prostate cancer [60]. Study endpoints included the safety, PK, biodistribution, antitumor effects and *ex vivo* antibody-dependent cellular cytotoxicity (ADCC) activity of intravenously administered huJ591. Each patient received four escalating

huJ591 doses of 10, 25, 50 and 100 mg, including approximately 2 mg (5 mCi) of ^{111}In-huJ591 used to image antibody localization. Treatment was administered at 3-week intervals.

A total of 15 patients were treated. All had bone and/or soft-tissue lesions. Approximately one-half of the patients received unlabeled and ^{111}In-labeled mAb concurrently, and the remaining patients received unlabeled mAb shortly (<10 min) before labeled mAb. One patient experienced an apparent hypersensitivity reaction following the first dose and was removed from the study. Treatment was well tolerated in the remaining patients, who completed the 4-dose course of therapy. The serum half-life was ~3.5 days at the 100mg dose level and lower at lower doses. While the highest uptake of radioactivity occurred in liver, hepatic saturation was observed at the 25mg dose. Notably, all patients showed tumor localization in at least one site, and both osseous and soft-tissue lesions were targeted efficiently. Post-treatment sera were tested for ADCC activity using peripheral blood mononuclear cells as effectors and LNCaP cells as targets, and modest ADCC activity was reported. One patient experienced a PSA decrease of >50%, and levels remained decreased for 6 months.

huJ591 in non-prostatic tumors: A phase 1 study was conducted to evaluate the safety, PK, immunogenicity and targeting of huJ591 in patients with advanced solid tumors of a type previously shown to express PSMA in the neovasculature [28]. Patients (n=27) received huJ591 at dose levels of 5 mg (n=3), 10 mg (n=9), 20 mg (n=3), 40 mg (n=6) or 40 mg (n=6). For each patient, the first dose included 1-2 mg (5 mCi) of ^{111}In-huJ591 for imaging and PK measurements. Patients in the 5 mg and 10 mg cohorts received 2 doses at weeks 0 and 2, while the other groups received 6 weekly doses. The study included 10 women and 17 men. Tumor types included kidney (n=10), colorectal (n=4), lung (n=3), bladder (n=3), pancreas (n=3), breast (n=3) and melanoma (n=1).

Treatment was well tolerated. Grade 3 events included infusion-related toxicity at the 10mg dose level and hypertension at the 80mg dose level. Patients treated with >10mg were premedicated to prevent infusion-related reactions. Liver accumulated the highest amount of radioactivity. However, 20 of 27 patients (74%) demonstrated mAb targeting of at least one area of known metastasis, and targeting of all known areas of metastatic disease was observed in 7 patients (35%). Tumor imaging was observed in 7/10 patients with kidney cancer, 4/4 colorectal cases, 3/3 lung cases, 1/3 bladder cases, 3/3 pancreatic cases, 2/3 breast cases and 1/1 melanoma patient. The serum half-life of huJ591 increased with dose and was ~60h at 80mg. No anti-huJ591 antibodies were observed. The trial was not designed to evaluate efficacy, and no objective tumor responses were observed. The authors noted that this trial provides the first successful demonstration of specific targeting of tumor neovasculature in humans [28].

MLN2704: MLN2704 (Millennium Pharmaceuticals, Inc., Cambridge, MA) is an antibody-drug conjugate that comprises huJ591 conjugated to the drug maytansinoid 1 (DM1, Immunogen, Cambridge, MA). DM1 [61,62] is a potent microtubule-depolymerizing drug derived from maytansine, a naturally occurring ansa macrolide [63,64]. DM1 is attached to lysine residues on huJ591 via a thiopentanoate linker. A disulfide bond within the linker is designed to be reduced intracellularly in order to release active DM1. MLN2704 contains on approximately 3-5 DM1 molecules per antibody [49].

In *in vitro* studies, MLN2704 eliminated PSMA-positive LNCaP cells with an IC50 of ~1.4 nM and a 44-fold selectivity index over PSMA-negative PC-3 cells (IC50 = 61 nM) [65]. MLN2704 was also examined in the CWR22 xenograft model in nude mice. Subcutaneous LNCaP tumors were treated with MNL2704 at doses of 5-60 mg/kg and schedules ranging from q3dX5 to q28dX5. The optimal regimen (60 mg/kg, q14dX5) delayed tumor growth by >100 days without apparent toxicity; however, no complete regressions were observed with any regimen in this model. MLN2704 also demonstrated encouraging activity in a novel model of osteoblastic bone metastasis based on 22RV1 cells [49].

Updates on clinical trials of MLN2704 were presented at the 2004, 2005 and 2006 annual meetings of the American Society of Clinical Oncology (ASCO) [66-68]. The trials examined the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of single [66] or multiple doses of MLN2704 [67,68] in patients with hormone-refractory prostate cancer (HRPC). Secondary endpoints included the PK of MLN2704 and its components, immunogenicity, serum PSA and tumor responses. In these studies, eligible patients had castrate levels of testosterone (<50 ng/dL) and progressive disease as defined by progressive tumor lesions, new bone metastases or a progressive rise in PSA. Major exclusion criteria included Karnofsky performance score < 60 and use of antiandrogen therapy within 6 weeks of enrollment. MLN2704 was infused intravenously over 2.5-3h.

In a first study [66], patients received MLN2704 at doses ranging from 18-343 mg/m^2. If no DLT was observed following the first dose, two additional doses were permitted at 4-week intervals, and responders were offered continued dosing at 4-week intervals until disease progression was observed. Twenty-three patients were treated in the study, including three at 264 mg/m^2 and six at 343 mg/m^2. Forty-eight percent of patients had received prior chemotherapy, which was taxane-based in 36% of patients. Baseline PSA values ranged from 5 to 807 ng/mL, with a median of 70 ng/mL, and 48% of patients had measurable disease at entry. Grade 3 adverse events included febrile neutropenia, lymphopenia, and increased alanine aminotransferase (ALT) levels, but an MTD was not established. The product had a 51-78h serum half-life at the higher dose levels. Both antibody and free DM1 were detected in plasma, suggesting possible deconjugation *in vivo*. No significant immunogenicity was observed. Of the patients receiving ≥264 mg/m^2 MLN2704, one achieved a confirmed partial response by RECIST and a sustained ≥50% reduction in PSA lasting 47 weeks, and a second patient experienced a ≥50% PSA reduction lasting 24 weeks [66].

A phase 1/2 study [67,68] was designed originally to test q1week and q2weeks schedules, and was amended to also examine q3weeks dosing as well as dosing on days 1 and 15 of a 42-day cycle (q6weeks schedule). A total of 62 patients were treated. All had received prior chemotherapy; 32 had received taxane-based therapy. Twenty-six patients had measurable disease at entry, and their median PSA was 59.8 ng/mL (range 3.9-5241 ng/mL). The q1week (60-165 mg/m^2), q2weeks (120-330 mg/m^2), q3weeks (330 and 462 mg/m^2) and q6weeks (330 mg/m^2) schedules were used to treat 12, 15, 18 and 17 patients, respectively. Transient elevations in hepatic transaminases and peripheral neuropathy were the main toxicities that limited treatment at 330 mg/m^2 q2weeks and 462 mg/m^2 q3weeks to 6 and 4 patients, respectively. Peripheral neuropathy was thought to be related to free DM1 released from the conjugate. PSA responses (≥50%) were not observed with q1week or q3weeks dosing but were reported for 2 of 6 patients treated with 330 mg/m^2 q2weeks and for 4 of 17 patients treated with the q6weeks schedule. The findings provide proof-of-concept for antibody drug-conjugate therapy of prostate cancer, but the therapeutic window was considered to be too narrow to support further development of the agent [68,69].

MDX-070: A fully human PSMA mAb, MDX-070 (Medarex, Bloomsbury, NJ), has been evaluated clinically as an unmodified antibody. Clinical testing was based in part on ADCC activity observed in preclinical studies of this IgG1 mAb. The clinical studies have examined the safety, PK and preliminary antitumor activity of escalating doses of mAb administered intravenously to patients with hormone-refractory disease. An initial study in 18 patients

examined single doses ranging from 0.1 to 10 mg/kg. In a follow-on study, patients received 1 (n=9), 5 (n=6) or 10 (n=15) mg/kg MDX-010 every two weeks for up to 4 doses. Patients with stable disease were eligible to receive additional treatment until disease progression was observed. In the two studies, possibly drug-related adverse events included one Grade 3 anorexia plus the following Grade 1 or 2 events: anorexia (n=2), fatigue (n=3), fever (n=4) and vomiting (n=2). The adverse events were not dose-limiting. No patient experienced a >50% decline in PSA or tumor response by RECIST. Six patients had stable disease and received at least one additional treatment. PK analyses and further enrollment in the 10 mg/kg multidose cohort were reported to be ongoing [70]. In addition, MDX-070 is being evaluated preclinically as part of an antibody-drug conjugate [71].

Pipeline antibody products: Compared to unmodified PSMA antibodies, PSMA antibody-drug conjugates have demonstrated greater preclinical proof-of-concept. Indeed, unmodified PSMA antibodies have served as negative controls in xenograft studies of PSMA antibody-drug conjugates [48,49]. In addition to the drug-conjugated PSMA antibodies described above, PSMA Development Company LLC (Tarrytown, NY) has reported preclinical results for a PSMA antibody-drug conjugate (PSMA ADC) [48]. PSMA ADC comprises a fully human, dimer-specific PSMA mAb linked to monomethylauristatin E (MMAE, Seattle Genetics, Inc., Bothell, WA), a dolastatin derivative that potently inhibits tubulin polymerization [72]. PSMA ADC incorporates a dipeptide linkage that is designed to maintain serum stability while maximizing intracellular drug release by human cathepsin B [73-77]. PSMA ADC was evaluated for antitumor activity *in vitro* and in a mouse xenograft model of androgen-independent disease. PSMA ADC eliminated PSMA-expressing cells with picomolar potency and >700-fold selectivity in cell culture. When used to treat mice with established human C4-2 tumors, PSMA ADC significantly improved median survival 9-fold relative to controls in the absence of apparent toxicity. Treatment effects were also manifest as significant reductions in serum PSA, and complete tumor regressions were observed in two of five animals treated with 6 mg/kg q4daysx6. PSMA ADC currently is being evaluated in preclinical toxicology studies.

CLINICAL TRIALS OF PSMA VACCINES

DCVax®-Prostate: DCVax®-Prostate (Northwest Biotherapeutics, Inc., Bothell, WA) consists of patients' autologous dendritic cells (DC) loaded with recombinant PSMA protein. Early clinical trials used HLA-0201-binding PSMA peptides designated PSM-P1 (LLHETDSAV) and PSM-P2 (ALFDIESKV), and periodic study updates have been provided [78-86]. For preparation of dendritic cells (DC), patients were leukophoresed, and peripheral blood mononuclear cells were isolated. Adherent cells were differentiated using granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4. Typically, DC were pulsed for 2h with 10 μg/mL of PSM-P1 and PSM-P2 prior to washing and infusion in normal saline. Intradermal injection also has been examined. Doses typically ranged from 3 million and 20 million DC, but doses of >40 million DC also were studied [78].

A phase 2 trial was conducted to assess the safety, immunogenicity and antitumor effects of the vaccine in men with recurrent disease following primary treatment. The trial studied six infusions of vaccine at 6-week intervals [80]. A total of 37 patients were enrolled. Treatment was well tolerated. One complete and 10 partial responders were reported based on a >50% reduction in PSA and/or RECIST criteria. A second phase 2 study was conducted in patients with hormone-refractory metastatic disease [81]. Vaccine therapy was again administered as six infusions at 6-week intervals. A total of 33 patients were enrolled. The number of DC per infusion varied from 5 to 24 million, with a mean of 17 million. Twenty-five patients completed one infusion cycle and were evaluated for response. Of these, six partial and 2 complete responders were reported. Responders included HLA-0201^+ and HLA-0201^- patients [81]. Immunomonitoring studies correlated clinical response with the baseline immune status of the patients as measured by skin test reactions to recall antigens and cytokine release by T cells following non-specific stimulation *in vitro* [79].

rsPSMA: Recombinant soluble PSMA (rsPSMA, PSMA Development Company LLC, Tarrytown, NY) is a purified protein that comprises the 707-residue extracellular domain of PSMA. The protein is expressed in stably transfected Chinese hamster ovary cells and purified to homogeneity under non-denaturing conditions. Purified rsPSMA retains the homodimeric conformation and enzymatic activity of native, cell-surface PSMA. In preclinical studies using Alhydrogel (alum) adjuvant, dimeric but not monomeric forms of rsPSMA elicited high titers of antibodies that recognized cell-surface PSMA [11].

A phase 1, open-label study was conducted to evaluate the safety and tolerability of ascending doses of rsPSMA protein adjuvanted with Alhydrogel. Eligibility criteria included biochemically progressive prostate cancer following definitive primary therapy but no radiographic evidence of disease progression. Both hormone-naïve and hormone-refractory patients were eligible. Patients received 50 μg (n=6) or 250 μg (n=8) rsPSMA with Alhydrogel at Weeks 1, 2, 3 and 7 via subcutaneous injection. Patients were followed for safety, immunogenicity and disease status for 26 weeks, with additional follow-up for patients who developed measurable immunity to PSMA.

Preliminary results of this trial have been reported [87]. The vaccine was generally well tolerated with no dose-limiting toxicity or serious adverse events observed. Injection-site reactions were minor and were independent of dose of rsPSMA. Two patients in the 250 μg dose group developed high-titered antibody responses to PSMA as measured by ELISA. The titers peaked at Week 13 and remained detectable through Week 38 in both cases. Additional studies are planned to evaluate rsPSMA in combination with other adjuvants.

Plasmid DNA and adenoviral prime-boost vaccine: Mincheff and colleagues reported findings from a clinical trial of a PSMA prime-boost vaccine. The trial was conducted in accordance with an investigational new drug application (IND) filed with the Bulgarian National Drug Institute [88]. In this study, the extracellular domain of PSMA was cloned into a modified version of the pcDNA3.1 expression vector (Invitrogen, Carlsbad, CA) or into a replication-defective (E1 and E3 deleted) adenoviral 5 (Ad5) vector (Quantum Biotechnologies, Toronto, CA). In each case, the expression construct included an immediate-early cytomegalovirus (CMV) promoter and a bovine growth hormone polyadenine (poly-A) tail. The expression construct did not contain a signal sequence, and the expressed PSMA was shown *in vitro* to be retained and proteosomally degraded intracellularly without reaching the cell surface [89]. A pcDNA3.1-based plasmid encoding full-length CD86 was used to provide a co-stimulatory signal, and a plasmid containing both ectodomain PSMA and full-length CD86 under separate CMV promoters and poly-A sequences was also studied.

The study enrolled 26 patients with relatively heterogeneous disease status and treatment history. Six patients had undergone radical prostatectomy prior to entry. Of these, 3 patients had biochemical recurrence with no detectable metastases and were treated with vaccine only, while 3 patients presented with bone or distant lymph node metastases and received combined hormone and vaccine therapy.

The other 20 patients had not undergone prior prostatectomy. Thirteen patients had advanced local disease but not metastatic disease. Of these, 2 patients received vaccine only, 7 patients received combined hormone and vaccine therapy, and 4 patients received combined hormone and vaccine therapy prior to surgery. The other seven patients had metastatic disease with no prior

Table 2. PSMA Plasmid and PSMA Adenovirus Dosing Groups

Group	Injections 1-2, q1weekX2	Injection 3 (DTH Challenge)	Injection 4	Injections 5-6, q1weekX2	Injection 7 (DTH challenge)	Booster Injection, q3weeks
1 (n=4)	pPSMA pCD86	pPSMA	Ad5-PSMA	pPSMA/CD86 GM-CSF	pPSMA	pPSMA/ CD86 or Ad5-PSMA
2 (n=6)	pPSMA GM-CSF	pPSMA	Ad5-PSMA	pPSMA/CD86 GM-CSF	pPSMA	
3 (n=3)	pPSMA pCD86 GM-CSF	pPSMA	Ad5-PSMA	pPSMA/CD86 GM-CSF	pPSMA	
4 (n=3)	pPSMA/CD86 GM-CSF	pPSMA	Ad5-PSMA	pPSMA/CD86 GM-CSF	pPSMA	
5 (n=7)	None	None	Ad5-PSMA	pPSMA/CD86 GM-CSF	pPSMA	
6 (n=3)	None	None	Ad5-PSMA	Ad5-PSMA	pPSMA	

pPSMA = plasmid encoding ectodomain PSMA; pCD86 = plasmid encoding CD86, pPSMA/CD86 = plasmid encoding both ectodomain PSMA and CD86; Ad5-PSMA = replication-defective adenovirus 5 vector encoding ectodomain PSMA.

prostatectomy: 3 patients received vaccine alone, and 4 patients received hormone and vaccine therapy.

Patients were immunized with one of six regimens as illustrated in Table **2**. Plasmids were injected intradermally in the naval area at a dose of 100 μg for injections 1-7. Delayed-type hypersensitivity (DTH) responses were assessed after the third and seventh injections (Table **2**). Booster injections of 100-800 μg were used, depending on the type of DTH response observed. Adenovirus was administered intradermally at a dose of 5×10^8 plaque-forming units (PFU), which was maintained constant throughout the study. Where indicated, patients also received 40,000 international units (IU) of GM-CSF protein (Leukine; Immunex, Seattle, WA) at the same site on day 2 post-immunization [88].

Immunizations were well tolerated. No serious adverse events or significant laboratory observations were reported. Positive DTH reactions were observed after the third immunization for 50%, 67% and 100% of patients in Groups 1, 2 and 3-4. All Group 1 patients had developed positive DTH responses following immunization with Ad5-PSMA. Similarly, all patients who received an initial immunization with Ad5-PSMA were reported to develop positive DTH responses. A follow-up serological study reported detectable PSMA antibodies in 21% of patients at baseline and in 12-50% of patients at longitudinal time points ranging from 3-36 months after initiation of vaccine therapy [90].

Syngeneic and xenogeneic PSMA DNA vaccines: In preclinical studies of DNA plasmid vaccines for cancer, unmodified self-antigens often fail to elicit measurable immunity, presumably due to self-tolerance mechanisms. However, in studies conducted initially with melanoma differentiation antigens [91,92], significant immune responses, rapid depigmentation and protection of syngeneic tumor challenge were elicited in mice immunized with DNA plasmids encoding xenogeneic (*e.g*., human) forms of the antigens. More recently, xenogeneic but not syngeneic forms of PSMA DNA vaccines were found to be capable of breaking tolerance in mice [93,94], and these findings provided a basis for human clinical studies of DNA plasmid vaccines encoding xenogeneic (mouse) and syngeneic (human) PSMA. In each case, PSMA expression was driven by an intron-containing CMV promoter [95].

A phase 1 dose-escalation trial was performed in HLA-A0201$^+$ patients with rising PSA in the presence or absence of clinical metastases [96,97]. DNA was delivered intramuscularly at 3-week intervals using a Biojector 2000 jet delivery device. Thirty-six patients were randomized to receive three vaccinations of plasmid DNA encoding full-length mouse or human PSMA at doses of 100 μg, 1,500 μg or 4,000 μg, followed by three vaccinations with PSMA from the other species. Approximately 75% of patients had non-castrate levels of testosterone upon entry, and the median Gleason score was 7. Vaccination was well tolerated at all dose levels. Anti-PSMA antibodies were not detected at high titers; however, an impact on PSA doubling time was reported at the highest dose level. An additional study of human and mouse PSMA DNA vaccines has been initiated in patients with renal cell carcinoma [98].

Pipeline vaccine products: Alphaviruses are positive-strand RNA viruses that can mediate high-level gene expression in mammalian cells via self-amplifying, subgenomic RNA molecules. Replication proceeds in the cytoplasm exclusively via double-stranded RNA intermediates that can induce interferon responses, and this characteristic endows alphavirus vaccines with self-adjuvanting properties. Recent preclinical studies have demonstrated potent immunogenicity and anti-tumor effects for alphavirus vectors encoding self-antigens. In comparative studies, self-antigens were superior to xeno-antigens when delivered via alphavirus vectors [99-101].

PSMA-VRP (PSMA Development Company LLC, Tarrytown, NY) is a single-cycle alphaviral particle that encodes full-length PSMA. PSMA-VRP is a vaccine replicon particle (VRP, AlphaVax, Inc., Cary, NC) that is based on an attenuated variant of Venezuelan equine encephalitis virus (VEEV) [102,103]. In preclinical studies conducted in mice, robust T and B cell responses to PSMA were elicited by a single injection of 2×10^5 infectious units (IU) PSMA-VRP, and responses were boosted following repeat immunizations. Anti-PSMA responses were detected following three immunizations with 10^2 IU and increased over doses ranging to 10^6 IU without overt toxicity. T and B cell responses had a Th1 phenotype, and the T cell responses were mapped to defined PSMA peptides. PSMA-VRP is completing GLP toxicology studies in anticipation of human testing.

CONCLUSIONS AND FUTURE DIRECTIONS

Recent clinical trials have provided significant proof of principle for PSMA as a target for prostate cancer and neovascular therapy. Radiolabeled mAbs to the ectodomain of PSMA have demonstrated efficient targeting of both bone and soft-tissue lesions in prostate cancer and neovascular targeting has also been demonstrated. Both radiolabeled and drug-conjugated mAbs have led to objective antitumor responses in a subset of patients as measured by PSA declines and/or tumor burden. Similarly, early-stage vaccine trials have shown that anti-PSMA immune responses can be generated without toxicity in prostate cancer patients. Notably, the studies have not identified any significant or unexpected target-related toxicity. The completed trials provide a firm foundation for exploration of optimized antibody and vaccine therapies, and new products are progressing toward the clinic. Clinical use of these products can be optimized both alone and in combination with other therapeutic approaches.

In addition to antibody and vaccine approaches, other PSMA-targeted treatment modalities are being examined within research environments. PSMA-directed "T-bodies" have been developed by fusing a single-chain PSMA mAb with signaling chains of the T-cell receptor, thereby retargeting T cells to destroy PSMA-positive tumor cells in both *in vitro* and *in vivo* settings [104,105]. Additionally, PSMA's enzymatic activity has been targeted for the development of prodrugs [106]. Such prodrugs are designed to be inactive until hydrolyzed by PSMA's carboxypeptidase activity at the site of the tumor, offering the potential for local antitumor activity while avoiding systemic toxicities. Lastly, RNA aptamers to PSMA have been used to deliver cytotoxic agents to tumors preclinically [107,108]. PSMA's unique pattern of expression and biological activities make this molecule an attractive target for exploring such novel treatment modalities.

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Preclinical findings for a PSMA-VRP vaccine are described in a recently published manuscript: Durso RJ, Andjelic S, Gardner JP, *et al.*, A novel alphavirus vaccine encoding prostate-specific membrane antigen elicits potent cellular and humoral responses. Clin Cancer Res 2007; 13: 3999-4008.

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