[Frontiers in Bioscience 19, 12-33, January 1, 2014]

Antibody-drug conjugates targeting prostate-specific membrane antigen

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1. ABSTRACT

Prostate-specific membrane antigen (PSMA) is an integral, non-shed membrane glycoprotein that is a well-characterized and clinically validated marker of prostate cancer. The expression profile and other biological properties of PSMA make it an attractive target for antibody-drug conjugate (ADC) therapy of prostate cancer, as well as a broad range of other tumors in which PSMA is abundantly expressed within the tumor neovasculature. PSMA-targeted ADCs have been developed using auristatin and maytansinoid drugs, and each ADC has undergone extensive preclinical testing and has completed phase 1 testing in men with advanced prostate cancer. The preclinical and clinical findings have largely substantiated the promise of PSMA as an ADC target. This report summarizes the completed studies, current status, and potential future directions for ADCs that target PSMA.

2. CELL-SURFACE MARKERS OF PROSTATE CANCER

Approximately 900,000 new cases of prostate cancer are diagnosed worldwide each year (1), placing this disease second only to lung cancer as the most common non-cutaneous malignancy of men. More than 70 percent of cases occur in developed countries. Clinically localized disease typically is treated with surgery or radiation. For recurrent, locally advanced, or metastatic disease, front-line systemic therapy involves androgen deprivation via leutinizing hormone-releasing hormone (LHRH) drugs or orchiectomy. Despite initial responses, nearly all tumors become resistant to androgen deprivation and ultimately progress despite continued castrate levels of serum testosterone, a state referred to as castration-resistant prostate cancer (CRPC). Metastatic CRPC (mCRPC) represents a lethal stage of disease for which there is no

cure. Bone metastases are common and are major contributors to morbidity and death. After decades of modest advances in care, the armamentarium for mCRPC has expanded markedly in recent years, leading to important improvements in patient care but also raising many questions with regards to the use, staging and potential combinations of agents (2). In addition, the survival benefit is limited to three to five months per treatment. Worldwide, metastatic prostate cancer continues to claim the lives of more than 250,000 individuals per year (1). There continues to be an urgent need for new therapies both to treat metastatic prostate cancer and to prevent the initial spread of disease beyond the prostate.

Amongst cell-surface molecules, prostate-specific membrane antigen (PSMA) is the only clinically validated marker of prostate cancer, and two ADCs targeting PSMA have entered human testing, as discussed in Section 6. Other proteins being targeted by investigational ADCs in prostate cancer include SLC44A4 and STEAP1. Both proteins have restricted patterns of expression in normal tissues. The anti-SLC44A4 ADC (ASG-5ME, Astellas and Seattle Genetics) is described elsewhere in this issue. STEAP1 (six-transmembrane epithelial antigen of the prostate 1) is highly expressed in prostate cancer (3) as well as other genitourinary, bone, breast, lung, and skin cancers (4-8). While other STEAP family proteins mediate iron and copper metabolism, STEAP1 lacks the canonical catalytic domain and is of unknown function (9). Pharmacokinetics and biodistribution were evaluated preclinically for anti-STEAP1 ADCs (10) that were prepared using either a native humanized IgG1 antibody or a THIOMAB construct containing two introduced cysteines (11). The THIOMAB-drug conjugate exhibited reduced clearance and deconjugation in male Sprague-Dawley rats (10). Phase 1 studies of an anti-STEAP1 ADC (RG7450, DSTP3086S: Roche) have commenced in prostate cancer (clinicaltrials.gov identifier NCT01283373). Both ASG-5ME and RG7450 contain monomethylauristatin E as the cytotoxic drug.

Additional prostate markers have been evaluated as ADC targets preclinically. TMEFF2 (TENB2, tomoregulin) is a chondroitin sulfate proteoglycan that is overexpressed in prostate cancer (12, 13). TMEFF2 (transmembrane protein with epidermal growth factor-like and two follistatin-like domains) showed early preclinical promise as an ADC target (14). However, despite preferential expression of TMEFF2 in cancer, expression in normal tissues was sufficiently high to accelerate ADC clearance through antigen-mediated uptake in preclinical studies (15, 16). ADAM17 (a disintegrin and metalloprotease domain family member 17) mediates proteolytic activation of tumor necrosis factor-α and Notch signaling. Overexpression of ADAM17 in prostate cancer has been associated with tumor invasiveness (17, 18). An anti-ADAM17 antibody mediated effective delivery of doxorubicin and other payloads to the interior of breast cancer cells *in vitro* (19). Finally, PSCA (prostate stem-cell antigen) is an androgen-regulated molecule whose expression increases with disease progression in prostate cancer (20, 21). PSCA is a glycosylphosphatidylinositol (GPI)-tethered surface protein with diverse and poorly understood roles in normal cells and cancer (22). An anti-PSCA antibody conjugated to maytansine demonstrated selective cytotoxicity towards PSCA-positive tumors *in vitro* and mediated regressions of established PSCA-positive tumors as large as 500 mm^3 (23). An unconjugated anti-PSCA antibody (AGS-1C4D4, Astellas) is being clinically evaluated in patients with CRPC (24, 25). However, to our knowledge, no ADC targeting PSCA, ADAM17, or TMEFF2 has been publically reported to be in active clinical development at this time.

While cell-surface markers such as PSMA and PSCA are associated with increasing disease severity, invasiveness, and progression, none is an established oncogenic driver in prostate cancer. Rather, androgen receptor (AR) is regarded as the primary driver of growth and progression of prostate cancer (26). AR binds androgen in the cytoplasm, migrates along microtubules, and then enters the nucleus, where it activates transcription of a host of genes that promote growth, survival, and metastasis (27). PSMA and PSCA are amongst the genes directly regulated by androgen (20, 28). This dependence has served as the basis for androgen-deprivation therapy of prostate cancer for decades. In addition, mCRPC often remains reliant on androgen signaling despite castrate levels of testosterone, as demonstrated by the survival benefit seen following treatment with potent antiandrogens, such as abiraterone and enzalutamide (29, 30). As an intracellular protein, AR does not provide a target for classical approaches to antibody-drug conjugate (ADC) therapy.

3. PSMA AS A TARGET FOR CANCER THERAPY

PSMA is an integral membrane glycoprotein with an archetypal type II topology. PSMA contains a short amino-terminal cytoplasmic region, a single transmembrane-spanning domain, and a large extracellular domain region. The full-length protein has 750 amino acids, ten N-glycosylation sites, and a molecular weight of approximately 100 kDa under denaturing conditions (31). Under native conditions, PSMA assembles into a non-covalent homodimer for which the ectodomain structure has been solved (32-34). The protein is not shed into the circulation to any significant level.

PSMA was first identified by the murine antibody 7E11 (35) and subsequently cloned from the LNCaP prostate cancer cell line (31). These pioneering studies demonstrated that PSMA is expressed abundantly in prostate adenocarcinoma relative to benign prostate and other normal tissues (31, 35, 36). Subsequent studies by numerous independent research groups have confirmed that PSMA expression is an essentially universal feature of prostate cancer (Table 1). Expression is widespread both in primary disease and the most common sites of metastasis, namely bone and lymph nodes. Limited information is available on PSMA expression in other sites of metastasis. Collectively, the published studies encompass nearly 4,000 tumor specimens at varying stages of disease, and the concordance of findings provides a reassuring level of target validation.

Table 1. Published reports of PSMA expression in prostate cancer

Tumor type	Reference												
	(108)	(52)	(60)	(53)	(48)	(51)	(54)	(151)	(152)	(153)	(154)	(155)	*Total*
Bladder carcinoma	5/5	7/13	6/6	1/1				167/167				10/10	*196/202 (97%)*
Breast carcinoma	1/1		5/6									11/11	*17/18 (94%)*
Colorectal cancer	6/6	3/19	5/5				110/130					12/12	*136/172 (79%)*
Gastric cancer							79/119						*79/119 (66%)*
Glioblastoma multiforme			1/1							29/32		4/5	*34/38 (89%)*
Liver, hepatocellular carcinoma												7/8	*7/8 (88%)*
Lung carcinoma	1/1		5/5	1/1								11/11	*18/18 (100%)*
Lymphoma												6/10	*6/10 (60%)*
Melanoma			5/5	1/1								7/10	*13/16 (81%)*
Metastases to liver from CRC or unspecified primary	1/1						16/19					2/2	*19/22 (86%)*
Metastases to lymph node from CRC							4/5						*4/5 (80%)*
Neuroendocrine carcinoma			5/5										*5/5 (100%)*
Ovarian carcinoma												10/10	*10/10 (100%)*
Pancreatic carcinoma			4/4	1/1								8/10	*13/15 (87%)*
Renal: clear cell carcinoma	9/9	8/17	11/11		20/20	16/21						11/11	*75/89 (84%)*
Squamous cell carcinoma											72/96		*72/96 (75%)*
Sarcoma			5/6						21/45			8/12	*34/63 (54%)*
Total	*23/23*	*18/49*	*52/54*	*4/4*	*20/20*	*16/21*	*209/273*	*167/167*	*21/45*	*29/32*	*72/96*	*107/122*	*738/906 (81%)*

76-100% **51-75%** **26-50%** **0-25%** **not tested**

Each of the studies examined protein expression by immunohistochemistry. The values indicate the (# PSMA-positive specimens)/(# total specimens examined).

Clinical validation of PSMA as a biomarker is provided by ProstaScint™ (capromab pendetide, Jazz Pharmaceuticals), which is an ^{111}In-labeled form of 7E11. ProstaScint™ is a diagnostic imaging agent used to detect potential occult soft-tissue metastases in newly-diagnosed or post-prostatectomy prostate cancer patients who are at high risk for metastatic progression (37). However, 7E11 binds an epitope located within the cytoplasmic region of PSMA and therefore may localize to sites of tumor necrosis *in vivo* (38), making this antibody less suitable for therapy than antibodies that bind the ectodomain of PSMA.

In primary disease, high PSMA expression has been associated with increasing tumor grade, pathological stage, aneuploidy, and disease recurrence after primary intervention (39-44). Overexpression of PSMA provides an independent predictor of disease recurrence. While nearly all tumors express PSMA, patients with more intense expression have significantly shorter PSA-free survival following primary therapy than do patients with moderate expression (40, 42, 44). In comparison with primary disease, metastases exhibit similar intensities of expression but possibly less uniformity in terms of the percentage of PSMA-positive tumor cells (42, 45-47).

PSMA also is expressed abundantly in the neovasculature of diverse non-prostatic tumors. Figure 1 illustrates the reciprocal pattern of PSMA expression in prostate and non-prostatic cancers. In prostate cancer, PSMA is expressed abundantly on the malignant epithelial cells that define prostate adenocarcinoma and is generally not expressed on the neovasculature. The exception is a minority of prostate cancers that have modest focal expression of PSMA in the neovasculature. In a range of non-prostatic tumors, the reverse is true. PSMA is expressed abundantly on the new blood vessels that supply the tumor, and is not expressed on the tumor cells themselves. Endothelial expression is luminal, facilitating access to antibody (48).

Numerous independent groups have confirmed intense and nearly universal expression in several important carcinomas, including those of bladder, breast, colon, liver, lung, ovarian, and renal origin (Table 2). Robust expression often is seen in other well-vascularized tumors such as glioblastoma multiforme and melanoma. Glioblastoma expression is notable in that an anti-PSMA antibody would not need to breach the blood-brain barrier in order to reach its therapeutic target. The immunohistochemical studies on neovascular PSMA are supplemented by clinical imaging studies, in which a radiolabeled anti-PSMA antibody demonstrated good localization across a range of non-prostatic tumors (49, 50).

Importantly, PSMA is not expressed on normal vasculature. Even within the same tissue section, PSMA is observed on tumor vasculature but not on adjacent normal blood vessels (51-54). The few reported instances of PSMA expression in non-neoplastic endothelium include cycling endometrium, granulation tissue and keloidal scars (55). Thus, whereas conventional anti-angiogenic or vascular-disrupting agents target proteins and pathways that are common to tumor and normal vasculature, PSMA may provide a means to selectively target therapy to tumor vasculature.

Table 2. Reports of neovascular PSMA expression in non-prostatic tumors

Tumor type	Reference												
	(108)	(52)	(60)	(53)	(48)	(51)	(54)	(151)	(152)	(153)	(154)	(155)	*Total*
Bladder carcinoma	5/5	7/13	6/6	1/1				167/167				10/10	*196/202 (97%)*
Breast carcinoma	1/1		5/6									11/11	*17/18 (94%)*
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Lymphoma												6/10	*6/10 (60%)*
Melanoma			5/5	1/1								7/10	*13/16 (81%)*
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Pancreatic carcinoma			4/4	1/1								8/10	*13/15 (87%)*
Renal: clear cell carcinoma	9/9	8/17	11/11		20/20	16/21						11/11	*75/89 (84%)*
Squamous cell carcinoma											72/96		*72/96 (75%)*
Sarcoma			5/6						21/45			8/12	*34/63 (54%)*
Total	*23/23*	*18/49*	*52/54*	*4/4*	*20/20*	*16/21*	*209/273*	*167/167*	*21/45*	*29/32*	*72/96*	*107/122*	*738/906 (81%)*

76-100% | 51-75% | 26-50% | 0-25% | not tested

Each of the studies examined protein expression by immunohistochemistry. The values indicate the (# PSMA-positive specimens)/(# total specimens examined).

PSMA's expression in tumor vasculature suggests a role in tumor angiogenesis; however, this role is poorly understood. Studies with PSMA-null mice indicate that PSMA enhances endothelial invasiveness by modulating activation of β_1 integrin. In these studies, PSMA was observed to promote both tumor and retinal angiogenesis (56, 57). The latter findings suggest a possible role for PSMA-targeted therapy in ocular disease; however, PSMA expression has not yet been reported in age-related macular degeneration or other retinal diseases in humans (58). Intriguingly, PSMA expression on tumor cells in prostate adenocarcinoma was positively associated with increased intratumoral vascularization (59). Further studies on the potential pro-angiogenic effects of PSMA are warranted.

PSMA expression in normal tissues has been evaluated at the level of mRNA and protein (35, 36, 52, 60-70). The findings are obscured in part by differences in methodologies, reagents, and standardization. In addition, confusion can arise from extrapolations of animal data to humans, as there are important species differences in PSMA expression. For example, PSMA is not expressed in normal prostate in rodents (65, 68, 71). Nevertheless, a few consistent themes have emerged. First, PSMA is expressed in a limited number of normal tissues. In addition to prostate, the most consistent sites of expression include the brain (astrocytes) (61, 70), small intestines (brush-border epithelium) (60, 66, 67, 70), and kidney (a subset of proximal tubules) (51, 53, 60, 70). Other potential sites include salivary gland (62, 70), liver (65, 69, 70), and spleen (65, 69). In rodents, PSMA is expressed in the peripheral nervous system (72), but data in humans are lacking. Second, expression in normal tissues is low. Relative to prostate cancer, expression is 10-fold lower in normal prostate and approximately 100-fold lower in other tissues. Finally, PSMA is undetectable in most human tissues.

PSMA is a biologically active molecule that functions as a zinc metalloprotease. Also known as glutamate carboxypeptidase II (GCP II, EC 3.4.17.21), PSMA cleaves C-terminal glutamate residues from various substrates (73). Intestinal PSMA facilitates absorption of folate. Dietary folates typically contain poly-gamma-glutamated moieties that hinder intestinal uptake. PSMA progressively removes γ-glutamate residues to yield folic acid, which is actively transported via the reduced folate carrier. An initial study (74) in a small group of elderly white Americans associated altered folate uptake with the most common PSMA genetic polymorphism (C1561T), which results in an H475Y amino acid substitution in the vicinity of the enzymatic site. Similar findings regarding alterations in folate metabolism have been reported in some but not all studies that have examined this issue (75-81), and further studies are needed to clarify the effect of PSMA genotype *v.* other genetic and environmental factors.

Following its initial cloning, PSMA was found to be identical to the brain hydrolase known as NAALADase (*N*-acetylated alpha-linked acidic dipeptidase) (82). Brain PSMA hydrolyzes the neuropeptide NAAG (*N*-acetlyaspartylglutamate) to liberate glutamate, which is the primary excitatory neurotransmitter in the human nervous

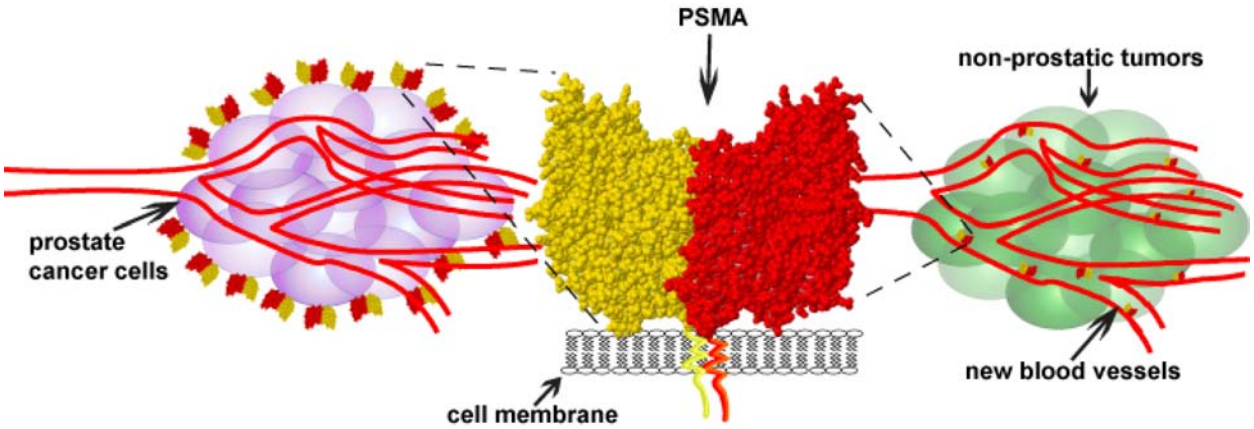

Figure 1. Cartoon illustrating the reciprocal pattern of PSMA expression in prostatic and non-prostatic tumors. In prostate cancer, PSMA is expressed on the epithelial-derived tumor cells but not on the neovascular endothelial cells. In non-prostatic tumors, PSMA is expressed on the endothelial cells of the tumor neovasculature, but is not expressed on the tumor cells themselves.

system. Both NAAG and glutamate mediate a broad range of physiological function related to learning, memory, and brain development. Their levels are tightly regulated under normal conditions, and imbalances are associated with diverse neurologic conditions. Because of this, PSMA/NAALADase inhibitors are being actively evaluated as potential neuroprotective agents, and there are excellent recent reviews of this topic (83-85).

The role of PSMA in tumorigenesis is less clear. Peptidase activity may contribute to invasiveness; however, opposing effects of PSMA on cell invasiveness have been observed in *in vitro* and *in vivo* models (56, 86-88). Folate is critical for both rapidly dividing cancer cells and endothelial cells; however, the major blood form is not polyglutamated and thus not a substrate for PSMA. Regardless of substrate, PSMA's carboxypeptidase activity releases glutamate, which recently has been shown to promote tumor growth and bone metastasis in prostate and other cancers via glutamatergic signaling pathways (89-91). Additional studies are needed to dissect the potentially complex roles of PSMA in tumor growth, metastasis, and angiogenesis.

While a mere 18 amino acids in length, the cytoplasmic domain of PSMA crucially affects its physiological activity. PSMA is rapidly internalized in the presence and absence of antibody (92-94). Endocytic trafficking occurs via both clathrin- and caveolae-dependent processes and is mediated by the five *N*-terminal amino acids (MWNLL) of PSMA (92, 95). The internalization machinery that recognizes this sequence appears to be broadly conserved across tissues and species as PSMA is rapidly internalized in human prostate, endothelial, and melanoma cells as well as murine, canine, and monkey cells (92, 94-97). PSMA's cytoplasmic domain also binds filamin A and the anaphase-promoting complex, providing a link with the cytoskeleton, integrin signaling pathways, and cell-cycle progression (56, 96, 97). The findings suggest a possible mechanistic role for PSMA in tumor progression independent of the molecule's enzymatic activity.

4. ANTIBODY-DRUG CONJUGATE CHEMISTRIES

PSMA targeting has been explored using ADCs employing both auristatin and maytansinoid drugs. Both drugs block microtubule function and cell division. Thus, both drugs and their corresponding ADCs offer a degree of selectivity for proliferating *v.* non-proliferating cells. Based on the regulatory approvals of Taxotere™ (docetaxel) (98) and Jevtana™ (cabazitaxel) (99) for mCRPC, microtubule inhibitors represent a validated approach to the therapy of prostate cancer. In addition to antimitotic effects, the activity of microtubule inhibitors in prostate cancer has been linked to inhibition of cytoplasmic transport of AR along microtubules to the nucleus (27, 100, 101). These considerations support the exploration of tubulin inhibitors as the cytotoxic components of ADCs for prostate cancer.

PSMA ADC (Progenics Pharmaceuticals; Figure 2), contains a fully human IgG1 mAb conjugated to vcMMAE (valine-citrulline monomethylauristatin E, Seattle Genetics) (94, 102). The mAb was prepared in transgenic Xenomice™ containing large contiguous fragments of the human heavy and kappa light chain immunoglobulin gene loci (103). The mAb binds PSMA with subnanomolar affinity and high selectivity, specifically recognizing an extracellular epitope presented by native dimeric PSMA but not monomeric forms of protein (32, 94, 102). The drug-linker and average drug-antibody ratio (4:1) are the same as those of ADCETRIS® brentuximab vedotin (Seattle Genetics) (104). To prepare the ADC, interchain disulfide bonds of the antibody are reduced under mild conditions to generate free cysteines according to published methods (105, 106). The reduced antibody and drug-linker are combined, enabling the antibody sulfhydryl groups to react with the maleimidocaproyl moiety of the drug-linker to form a

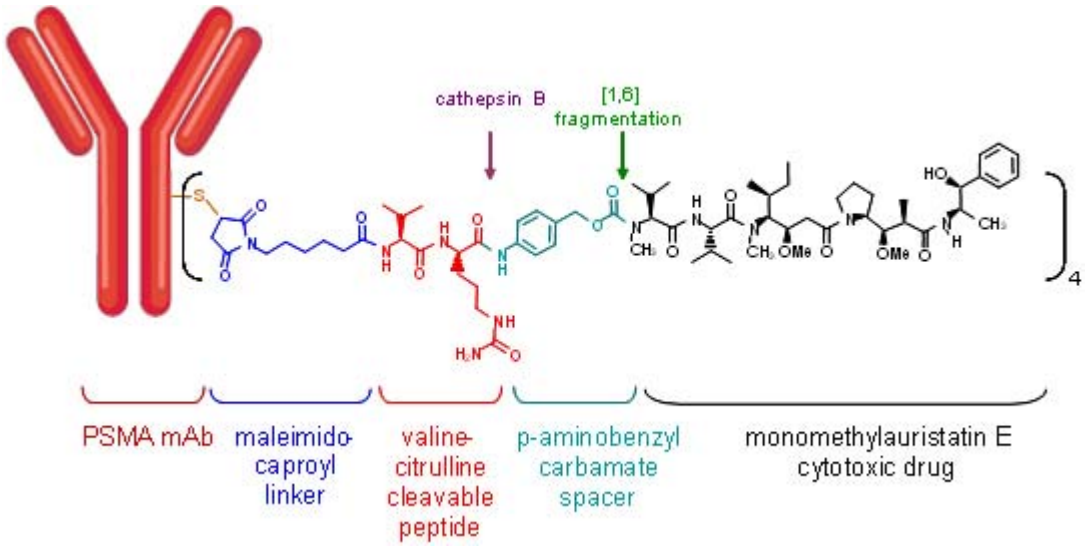

Figure 2. Structure of PSMA ADC.

thioether linkage. The valine-citrulline dipeptide is designed to be stable in blood but efficiently cleaved at the citrulline carboxyl group by lysosomal enzymes such as cathepsin B. Peptide cleavage triggers self-elimination of the *p*-aminobenzyl carbamate spacer through a 1, 6 elimination process to liberate free MMAE, a potent inhibitor of tubulin polymerization. Excellent descriptions of vcMMAE and other auristatin-based drug-linkers can be found elsewhere (107).

MLN2704 (Figure 3) was based on a deimmunized form of the murine mAb J591. Developed by Dr. Neil Bander and his colleagues at the Cornell-Weill School of Medicine, J591 binds a linear epitope within the extracellular region of PSMA with nanomolar affinity (93, 108). Murine J591 was deimmunized by first preparing a chimeric monoclonal antibody (mAb) consisting of the murine J591 variable regions genetically fused to human IgG1,κ constant regions. For deimmunization, potential B-cell and T-cell epitopes were identified according to published procedures, and framework residues within the J591 variable were replaced with germline human amino acids (109). MLN2704 was prepared in a two-step procedure. Deimmunized J591 (HuJ591) was first modified by reaction at pH 6 with N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), resulting in attachment of a thiopentanoate group to antibody lysine residues. SPP-modified antibody was then reacted with drug maytansinoid 1 (DM1, ImmunoGen), resulting in a disulfide linkage between the drug and antibody (Figure 3). The disulfide linkage is sterically hindered via a methyl group on the adjacent carbon of the SPP linker (110) and was designed to release DM1 under the reducing conditions of the cell interior. The average substitution was three to four drugs per antibody (109). An analog of a naturally occurring ansa macrolide, DM1 is a potent microtubule-depolymerizing drug whose properties have been well described in prior publications (111, 112).

5. PRECLINICAL FINDINGS

5.1. PSMA ADC

An initial report described the internalization, *in vitro* cytotoxicity, and xenograft efficacy of PSMA ADC and its parent mAb (94). Internalization of ^{111}In-labeled parent mAb was examined on C4-2 cells, an androgen-independent prostate cancer cell line (113). Over one-half of bound mAb was internalized within two hours. The remaining 40 percent of bound mAb remained associated with the cell surface through 30 h, possibly through recycling of PSMA. *In vitro* cytotoxicity was tested against C4-2, LNCaP and 3T3-PSMA cells, each of which express greater than 10^5 copies of PSMA per cell. IC50 values ranged from 65 to 210 pM. These IC50 values were approximately 1,000-fold lower than those for a nonbinding vcMMAE-conjugated antibody used as a control. PSMA ADC was also evaluated in a disseminated C4-2 mouse xenograft model in which tumors were implanted intramuscularly. Animals were randomized to treatment groups according to serum PSA levels 14 to 17 days post-implantation. Intravenous treatment regimens of 2 or 10 mg/kg Q4Dx3 and 3 or 6 mg/kg Q4Dx6 were examined in separate studies. Efficacy endpoints included overall survival and changes in serum PSA. Each schedule resulted in significant dose-dependent antitumor activity by each of the endpoints analyzed. The 10 mg/kg Q4Dx3 dose resulted in significant PSA decreases relative to baseline, while the 2 mg/kg dose significantly slowed the otherwise rapid rise in PSA (approximately 100-fold increase in two weeks) seen in the absence of treatment. The most durable responses were seen with the Q4Dx6 regimen, where the 6 mg/kg dose led to complete tumor regressions in two of five animals lasting to 500 days post-implantation. No weight loss or other signs of overt toxicity were associated with treatment with PSMA ADC (94).

In a second publication (102), PSMA ADC, a nonbinding vcMMAE-conjugated antibody, and free

Table 3. Summary of xenograft efficacy studies conducted with PSMA ADC

Tumor	Site	Outcomes	Effective Doses
C4.2	IM	Decreased PSA and improved survival	2, 10 mg/kg Q4Dx3 3, 6 mg/kg Q4Dx6
C4.2	SC	Decreased PSA, tumor regressions and improved survival	1, 3, 6 mg/kg 2X/W for 3W 10 mg/kg Q6Dx2 2, 8 mg/kg single dose
C4.2 docetaxel relapse	SC	Tumor regressions and improved survival	6 mg/kg weekly
LuCaP96CR	SC	Tumor regressions and improved survival	1.5, 3, 6 mg/kg Q1Wx4
LNCaP	SC	Tumor regressions	1, 5 mg/kg single dose
LuCaP 77	SC	Tumor regressions	5, 10 mg/kg single dose
LuCaP 70	SC	Tumor growth inhibition	6 mg/kg single dose

MMAE were compared for cell-killing activity *in vitro* against a panel of prostate cancer cell lines that varied according to expression of PSMA. Free MMAE exhibited consistent activity across all cell lines; IC50 values ranged from 0.3 to 1.4 nM. PSMA ADC and the control ADC showed equivalent activity against PSMA-negative PC-3 and DU145 cell lines. PSMA ADC showed approximately 100-fold selective cytotoxicity towards CWR22rv1 cells that express 10^4 molecules of PSMA per cell. Maximum potency and selectivity of PSMA ADC were observed for cells with greater than 30,000 molecules of PSMA per cell. In this study, the selectivity of PSMA ADC towards PSMA-positive and PSMA-negative cells ranged up to 9,000-fold. Studies with mutated forms of PSMA indicated that the cytotoxicity of PSMA ADC was also dependent on PSMA internalization and proper glycosylation and folding of the antigen (102).

In vivo activity was evaluated in a novel mouse xenograft model of acquired docetaxel resistance *in situ*. In this model, animals with 100-200 mm3 subcutaneous C4-2 tumors were randomized to receive an initial course of treatment with vehicle or docetaxel at its maximum tolerated dose in the tumor-bearing animals. Weekly intravenous doses of 2 mg/kg docetaxel had significant antitumor activity, resulting in greater than 80 percent tumor growth inhibition relative to vehicle initially. Animals remained on docetaxel for as long as tumor growth was controlled, and docetaxel controlled tumor growth throughout the course of the study in some animals. However, most animals (72 percent) had tumors that progressed to greater than 400 mm3 while on docetaxel, and these animals were then randomized to receive continued docetaxel or weekly 6 mg/kg PSMA ADC intravenously. The mean tumor volume upon initiation of treatment with PSMA ADC was 515 mm3, with some tumors ranging to greater than 700 mm3. Despite this tumor burden and the taxane-refractory nature of the disease, PSMA ADC treatment resulted in potent tumor regressions in all animals, and all animals randomized to PSMA ADC survived to the end of the study. In contrast, animals that were randomized to receive continued docetaxel had progressive disease and poor survival outcomes. There was no apparent toxicity associated with PSMA ADC treatment (102).

Table 3 summarizes xenograft efficacy studies performed using PSMA ADC. Androgen-independent C4-2 cells have been used in a variety of settings given their favorable growth properties *in vitro* and *in vivo*. Both disseminated intramuscular and bulky subcutaneous models have been examined, and the subcutaneous model was adapted to the docetaxel relapse setting, as described above. In addition, three other tumors of varying genetic background (LNCaP, LuCaP 77 and LuCaP 70) were screened in single-dose efficacy studies. Efficacy readouts included survival, tumor volume and serum PSA levels. As indicated in Table 3, treatment with PSMA ADC led to significant survival benefit, tumor regressions, and PSA decreases in each of the models with the exception of LuCaP 70. In the latter model, treatment was associated with tumor growth inhibition but not frank tumor regressions. In cases that examined different doses of PSMA ADC, antitumor effects typically were first seen at doses of one to two mg/kg. PSMA ADC exhibited potent antitumor activity that was not observed for a control nonbinding ADC used at equivalent doses.

5.2. MLN2704

The J591 antibody also rapidly internalizes into prostate cancer cells that express PSMA (93). MLN2704 was compared for cytotoxicity *in vitro* against the LNCaP and PC-3 prostate cancer cell lines. The respective IC50 values for PSMA-expressing and non-expressing cells were 1.4 nM and 61 nM, for an *in vitro* selectivity index of 44-fold (114). The cytotoxic effects of the conjugate were also measured against three different 22Rv1 clones that had been engineered to express firefly luciferase (22Rv1luc). Across the three clones, IC50 values ranged from approximately 0.2 to 20 nM and loosely correlated with the level of PSMA expression (114).

In vivo efficacy was examined in the CWR22 model. Tumors were implanted subcutaneously and allowed to grow to a volume of approximately 200 mm^3 prior to treatment. An initial set of studies examined five repeat doses of approximately 13 mg/kg administered at intervals of 3, 7, 14, 21, or 28 days. At this dose, none of the regimens resulted in significant tumor regressions, and the main endpoint was the time to tumor progression following the initiation of therapy. By this metric, the 14-day dosing interval was optimal, and additional dose-

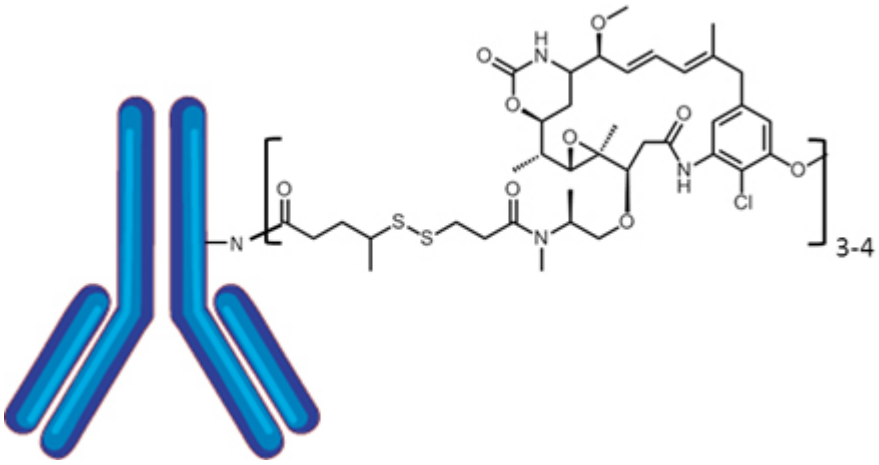

Figure 3. Structure of MLN2704.

ranging studies were conducted using a Q14dx5 regimen. Dose-dependent antitumor efficacy was observed over the dose range of 5 to 60 mg/kg. The 60 mg/kg dose regressed tumors to non-palpable levels at the end of treatment in most animals; however, all tumors regrew approximately 6 weeks after cessation of treatment. No overt toxicity was noted for any of the treatment regimens.

Plasma concentrations of MLN2704, total antibody, and deconjugated antibody were measured by ELISA. Deconjugated antibody was measured following depletion of MLN2704 from the plasma sample with an anti-DM1 antibody. Plasma concentrations of free DM1 were not reported. Mean terminal plasma half-lives were 39.2 h, 99.9 h, and 268 h for MLN2704, total antibody and deconjugated antibody, respectively. Plasma concentrations of deconjugated antibody exceeded those of MLN2704 at 48 h and were several-fold higher at 96 h. By 7 days and at later timepoints, minimal amounts of MLN2704 were detected, and deconjugated antibody was the predominant species.

MLN2704 also was tested in a mouse xenograft model of bone metastases. The model utilized the 22Rv1luc clone that was most sensitive to MLN2704 *in vitro*, as described above. For tumor implantation, a small hole was drilled into the tibiae of *scid* mice, into which the 22Rv1 cells were injected. Tumor growth was monitored by bioluminescence imaging, and bone lesions were imaged by micro-computed tomography. In the study, MLN2704 treatment (13 mg/kg Q3dx5) resulted in approximately 80 percent tumor growth inhibition relative to vehicle treatment 48 days post-implantation. MLN2704 treatment also prevented the formation of osteoblastic lesions detectable by micro-computed tomography (114).

5.3. Lessons from preclinical studies

PSMA facilitated rapid cellular internalization of the antibody component of both ADCs, with the majority of bound antibody internalized within a few hours. Potent and selective *in vitro* cytotoxicity was observed that varied according to the ADC construct and level of PSMA expression. For one ADC, the threshold level of PSMA expression for selective cytotoxicity was 10,000 copies of PSMA per cell. The relatively modest threshold may reflect PSMA's rapid rate of internalization and intracellular trafficking. *In vivo* efficacy was observed against diverse and difficult-to-treat xenograft tumors over a range of doses. Potent antitumor effects were achieved in the absence of apparent toxicity to the animals. Collectively, the findings provide a solid preclinical foundation of support for PSMA as an ADC target.

6. CLINICAL DEVELOPMENT STATUS

6.1. PSMA ADC

The first-in-humans study of PSMA ADC was completed recently (Study PSMA ADC 1301, clinicaltrials.gov identifier NCT01414283), and final study data are not yet available. This multi-dose, dose-ascending phase 1 study was designed to assess tolerability, pharmacokinetics, and antitumor effects across a range of doses and to identify the maximum tolerated dose (MTD) and dose-limiting toxicity. The study was conducted in subjects with progressive mCRPC who had received prior taxane-based chemotherapy. All subjects had failed docetaxel treatment. In the higher dose cohorts, a significant fraction had also failed abiraterone. Subjects received up to four doses of PSMA ADC by intravenous infusion at three-week intervals. At the conclusion of the initial 12-week period, subjects were offered enrollment into an extension study. The 1301 core and extension studies were conducted at sites in the United States exclusively.

The 1301 trial enrolled 52 subjects at doses ranging from 0.4 to 2.8 mg/kg. Intermediate doses levels were 0.7, 1.1, 1.6, 1.8, 2.0, 2.2, and 2.5 mg/kg. A dose of 2.5 mg/kg was determined to be the MTD, and neutropenia was found to be the predominant dose-limiting toxicity (DLT). Preliminary results from this trial were presented recently (115, 116), and these findings are summarized here. Final data are being collected and will be reported separately.

Phase 2 testing of PSMA ADC was initiated in September 2012 (clinicaltrials.gov identifier NCT01695044). PSMA ADC 2301 is a phase 2, open-label, single-arm study to assess the anti-tumor activity and tolerability of PSMA ADC in subjects with metastatic castration-resistant prostate cancer. Subjects must have received at least one, but no more than two cytotoxic chemotherapy regimens, one of which must have contained docetaxel. Subjects will receive up to eight 2.5 mg/kg doses of PSMA ADC by intravenous infusion at three week intervals. Key endpoints include antitumor response and changes in tumor assessments (RECIST 1.1 criteria), changes in circulating serum PSA, and changes in circulating tumor cells. No data are currently available from this study.

6.2. MLN2704

The MLN2704 clinical program has been summarized in a publication and a series of meeting presentations (117-121) (clinicaltrials.gov identifiers NCT00052000, NCT00070837, NCT00058409). Galsky *et al.* (121) reported on a group of subjects that received either low single doses (18 to 71 mg/m^2) or were eligible to receive higher repeat doses (92 to 343 mg/m^2) at four-week intervals. The broader phase 1/2 program also examined dosing according to one-, two-, three-, and six-week cycles. The six-week cycle examined dosing on days 1 and 15 followed by a four-week rest period.

Study objectives included determining the MTD for a given dose schedule, pharmacokinetics, and disease response for different dose regimens. Entry criteria included progressive mCRPC; adequate hematological, liver and renal function; and no brain metastases or peripheral neuropathy of grade 2 or higher at baseline. Prior use of taxane-based chemotherapy was not an inclusion criterion as the phase 1/2 program spanned the approval of docetaxel for mCRPC in the United States. The MLN2704 studies were conducted in the United States exclusively.

Approximately 85 subjects were treated in the MLN2704 phase 1/2 program. Twelve subjects received weekly MLN2704 doses of 60, 84, 118, or 165 mg/m^2 (N=3/group). The Q2W schedule examined 120, 168, 236, or 330 mg/m^2 in cohorts of three (low dose groups) to six (high dose group) subjects. Enrollment to the Q3W schedule included 14 subjects at 330 mg/m^2 and 4 subjects at 462 mg/m^2. Twenty-three subjects were treated with the single-dose or Q4W regimen, including 6 subjects at the highest dose of 343 mg/m^2 Q4W. Finally, seventeen subjects were treated according to a 330 mg/m^2 Q6W regimen. MLN2704 was administered by intravenous infusion over 2.5 h (121).

The mean age was 66 years (range 53 to 81 years) for the 23 subjects treated with the single-dose or Q4W regimens, and 68.5 years (range 52 to 84) for the 62 subjects treated according to other regimens. The respective median PSA values at baseline were 138.3 ng/mL (range 4.3 to 947.2 ng/mL) and 59.8 ng/mL (range 3.9 to 5240.9 ng/mL). Median Karnofsky performance status was 90 across all 85 subjects. Prior use of docetaxel was reported in 35 percent of subjects treated with the single-dose or Q4W regimens and 52 percent of subjects treated with other dose schedules.

As described below, PSA responses and other indications of antitumor activity were observed at varying frequencies with different dose schedules. However, based on the nature of the toxicity profile observed, the therapeutic window was considered to be too narrow to support further development, and the product was discontinued following conclusion of the phase 1/2 studies.

7. CLINICAL PHARMACOLOGY

7.1. PSMA ADC

PK data were collected following the first and third treatment cycles for subjects in each of the first three dose groups. Analytes measured were PSMA ADC, Total Antibody (sum of ADC and any unconjugated antibody) and free MMAE. PSMA ADC and Total Antibody were measured in serum using validated enzyme-linked immunosorbent assays (ELISAs). The ELISAs for PSMA ADC and Total Antibody utilized formats involving capture with anti-MMAE and anti-idiotypic antibodies, respectively. Plasma concentrations of free MMAE were measured in a validated assay based on high pressure liquid chromatography/tandem mass spectrometry. No appreciable accumulation of any analyte or differences in PK metrics were observed between the first and third treatment cycles for the first three dose cohorts, and PK data were collected following the first cycle only in subsequent dose groups. Preliminary data for cycle 1 are summarized below.

Exposure to PSMA ADC increased with dose in an approximately linear fashion over the range of doses tested. Mean clearance was 1.6 mL/(h•kg) at the top dose of 2.8 mg/kg. There was no clear trend between clearance and dose over the range of doses tested. The mean terminal serum half-life was approximately 2 days at 2.8 mg/kg. PSMA ADC serum concentrations of 100 ng/mL (approximately 650 pM, several-fold above the *in vitro* IC50) were sustained for 14 to 21 days after treatment with doses of 1.8 mg/kg and higher.

Mean serum concentrations of free MMAE remained below 10 ng/mL at all dose levels. Mean MMAE concentrations peaked between two and four days after treatment with the higher doses of PSMA ADC. MMAE was cleared with a serum half-life of approximately three days. The MMAE concentration-time profile and PK metrics following treatment with PSMA ADC were comparable to those observed following treatment with brentuximab vedotin (104).

Exposures to Total Antibody were approximately two-fold greater than the exposure to PSMA ADC. Other PK metrics for Total Antibody also closely tracked those for the ADC. Overall, the PK findings for free MMAE, Total Antibody, and PSMA ADC indicate that this agent is not rapidly hydrolyzed to release free MMAE in humans. More complete PK data for PSMA ADC and its chief

metabolites will be reported separately. According to preliminary data from the study, no subject developed measurable level of antibodies to PSMA ADC.

7.2. MLN2704

PK data were reported following the first MLN2704 treatment cycle of the Q4W regimen (121). Analytes included MLN2704, Total Antibody, Deconjugated Antibody, and free DM1. ELISA assays were also used to quantitate concentrations of MLN2704, Total Antibody, and Deconjugated Antibody. MLN2704 concentrations were quantitated using an anti-idiotypic antibody for capture followed by detection with an anti-DM1 antibody conjugated to horseradish peroxidase (HRP). Total Antibody was captured using an anti-idiotypic antibody and then detected using an HRP conjugate of an $F(ab')_2$ to human Fc. Concentrations of Deconjugated Antibody were determined by first depleting MLN2704 from the sample with an anti-DM1 antibody and then performing the assay for Total Antibody. Free DM1 (DM1-SH) was measured using liquid chromatography/tandem mass spectrometry after adding 4-4'-dipyridyl disulfide (PDS) to the serum specimen to form a stable DM1-PDS conjugate. Although different analytes were tested, concentration-time profiles were provided for MLN2704 and free DM1 only, and detailed PK metrics were reported for MLN2704 only.

Mean PK metrics for ADC and free drug were reported. Exposure to MLN2704 increased linearly with dose at doses greater than 120 mg/m^2 (approximately 3 mg/kg). At 120 mg/m^2 and lower doses, serum concentrations of MLN2704 did not remain above the lower limit of quantitation (750 ng/mL) sufficiently long to adequately sample the terminal elimination phase. MLN2704 was detectable in serum for up to 7 days after treatment with 120 mg/m^2 and for up to 21 days after treatment with 343 mg/m^2. Regression analysis revealed a trend towards decreased clearance of MLN2704 with increasing total dose expressed on a milligram basis. Mean clearance was 0.0448 L/(h•m^2) [approximately 1.2 mL/(h•kg)] at the top dose level of 343 mg/m^2. At this dose, the mean terminal serum half-life of MLN2704 was 2.5 days, as compared with an apparent half-life of 1.2 days at 120 mg/m^2 (120). Given the observation that clearance of MLN2704 decreased with increasing dose, it bears noting that the highest dose tested of MLN2704 (343 mg/m^2 or approximately 9 mg/kg) is approximately three times the top dose of PSMA ADC (2.8 mg/kg)

The clearance observed for MLN2704 in humans is consistent with preclinical findings for this compound in monkeys (109) and with studies of radiolabeled forms of huJ591. Serum half-lives of 0.5 to 3.5 days were observed for ^{111}In, ^{177}Lu- and ^{90}Y-labeled HuJ591 in patients with prostate cancer and other solid tumors (49, 50, 122-124). Clearance of radiolabeled HuJ591 decreased with increasing dose of antibody. Whole-body imaging consistently identified the liver as a major site of accumulation of HuJ591 (49, 50, 122-124). It was postulated that the liver serves as a saturable sink for HuJ591 with incomplete saturation at 100 mg antibody (124).

Free DM1 concentrations were reported for MLN2704 doses of 120 to 343 mg/m^2. Mean serum concentrations of free DM1 peaked between two and six hours following infusion, exceeding 200 ng/mL at the 343 mg/m^2 dose level. Mean peak DM1 concentrations of approximately 100 ng/mL were observed following dosing with 120 to 264 mg/m^2. The patterns of appearance of free DM1 and unconjugated antibody were considered to reflect deconjugation of MLN2704 (121), and the rate of onset is suggestive of deconjugation within the central compartment. MLN2704 was non-immunogenic in this study as determined by the lack of anti-product antibodies observed in any subject.

7.3. Lessons from clinical pharmacology studies

Perhaps the most notable pharmacology finding relates to the magnitude and consistency of the serum half-lives of PSMA antibodies and their conjugates. As noted above, serum half-lives of 2 to 3 days were observed for each ADC as well as radiolabeled forms of HuJ591. These findings are indicative of a class effect for anti-PSMA antibodies. Overall, the PK metrics and profiles suggest that antigen-mediated clearance may impact the PK of antibodies to PSMA over the range of doses tested in these studies (up to 343 mg/m^2 or approximately 9 mg/kg). Potentially, low levels of PSMA expression (*e.g.*, below a threshold level required for significant cytotoxicity) in one or more tissues could provide a sink for antibody, especially given the rapid internalization and recycling of cell-surface PSMA.

The observed serum half-life provides a rationale for exploring dosing schedules other than Q3W, as was done in the MLN2704 program. For MLN2704, the most promising regimen was felt to be Q2W (117), which may provide the optimal balance between antitumor effects and recovery from toxicity for this agent. Anti-product antibodies were not observed to either ADC in these studies, and thus it appears unlikely that immunogenicity will significantly limit the use of such agents in men with advanced prostate cancer.

8. CLINICAL SAFETY PROFILE

8.1. PSMA ADC

The PSMA ADC 1301 trial was designed primarily to assess safety across the dose-escalation range and to determine a maximum tolerated dose (MTD) for future studies. Safety assessments included periodic physical examinations, adverse events reporting, standard clinical laboratory tests, and electrocardiograms. Dose-limiting toxicity (DLT) was assessed during the first treatment cycle of each cohort. Dose escalation to the next cohort was allowed to proceed if DLT was observed in none of the first three subjects or no more than one of the first six subjects treated in a cohort.

From a starting dose of 0.4 mg/kg, dose escalation proceeded to 2.8 mg/kg. Doses of 2.5 mg/kg and below were generally well tolerated. At 2.8 mg/kg,

neutropenia was the predominant dose-limiting toxicity with one death due to neutropenic sepsis. PSMA ADC did not appear to appreciably affect other measures of marrow function. A transient asymptomatic increase in liver function parameters was seen in one subject at this dose level. Neutropenia and transient increases in transaminases are expected side effects of treatment with vcMMAE-based ADCs at this dose (104). .

One subject treated with 1.8 mg/kg experienced acute pancreatitis that progressed to death. Subsequent subjects were monitored closely for serum amylase and lipase, and no significant elevations were associated with further treatment at 1.8 mg/kg or higher doses. Pancreatic toxicity has not been identified as an adverse reaction to MMAE, and there is no known expression of PSMA in the pancreas. Thus, while a causal relationship cannot be excluded, the evidence suggests that the pancreatitis may have been due to factors other than treatment with study drug.

Peripheral neuropathy of varying severity, reversible following discontinuation of drug, was reported in a small number of subjects in the 1301 trial, typically those with a prior history of taxane-related neuropathy and usually after receiving several repeat doses of PSMA ADC. There was no suggestion of excess neuropathy relative to other ADCs that contain MMAE. More detailed safety information will be provided after final data are available.

8.2. MLN2704

The main toxicities observed for MLN2704 were neurologic, hepatic and hematologic in nature. The balance of toxicities varied according to the dosing schedule. For the Q1W schedule, peripheral neuropathy (grade 2 or lower) was reported in each of the three subjects treated at the top dose of 165 mg/m^2, and dose escalation was discontinued prior to establishment of an MTD. Grade 2 peripheral neuropathy was also the main toxicity that limited dose escalation beyond 330 mg/m^2 Q2W. Transient grade 2/3 elevations in hepatic transaminases were also observed in three of six subjects treated with 330 mg/m^2, and further dose escalation at Q2W was not considered appropriate. Of the four subjects treated with 462 mg/m^2 Q3W, there were grade 3 elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and one grade 2 peripheral neuropathy. Peripheral neuropathy was the main safety finding in the fourteen subjects treated at 330 mg/m^2 Q3W, with grade 2 or higher toxicity seen in the majority of subjects. Of the nine subjects treated with 264 or 330 mg/m^2 Q4W, drug-related grade 3 findings included febrile neutropenia, lymphopenia, and AST elevation, each in one subject. In addition, five of six subjects treated with 343 mg/m^2 experienced peripheral neuropathy characterized by numbness, paresthesia, and dysesthesia in the hands and feet. Toxicities observed in the seventeen subjects treated with 330 mg/m^2 Q6W included grade 3 AST/ALT elevations in one subject, grade 3 peripheral neuropathy in two subjects, and grade 2 neuropathy in six subjects. The onset of neuropathy typically was 6 weeks or later. There was no obvious association between the occurrence of neuropathy and baseline characteristics. The condition typically remained stable or improved in weeks to months after the discontinuation of treatment. The neuropathy was hypothesized to be related to free DM1.

Fatigue, nausea and diarrhea were the most common toxicities of any severity. Most cases were grade 2 or lower, were manageable, and did not interfere with daily activities. Infusion-related reactions were infrequent and manageable. No hypersensitivity reactions were reported. Overall, the pattern of toxicities was thought to reflect free DM1 released following its release from the conjugate in the circulation and/or following nonspecific catabolism of the antibody in tissues such as the liver.
It bears noting that the MLN2704 doses examined in these studies (*e.g.*,, 330 and 462 mg/m^2 Q3W) were higher than the MTD established in solid tumors for cantuzumab mertansine (235 mg/m^2 Q3W), an ADC with the same DM1-SPP drug-linker (125). The high doses of MLN2704 may be the most important factor for the incidence of neuropathy and other clinical toxicity findings.

8.3. Lessons from clinical safety findings

For both PSMA ADC and MLN2704, there was no obvious target-related toxicity that could be attributed to expression of PSMA in any organ or tissue. That is, there was no obvious pattern of gastrointestinal, CNS, or renal toxicity that theoretically could derive from PSMA expression within normal tissues of the small intestines, brain, or kidney. The lack of toxicity likely reflects lower expression of PSMA and its accessibility to antibody in these normal tissues. For example, the blood-brain barrier may limit access of ADCs to brain PSMA. Similarly, in small intestine and proximal tubules of the kidney, PSMA expression is luminal and thus not in direct contact with the circulating ADC. In addition, the cumulative data argue against any excess peripheral neuropathy over that attributable to circulating levels of free drug released from the conjugate. The overall safety profiles of PSMA ADC and MLN2704 are consistent with that of their respective free drugs, and thus management of patients treated with anti-PSMA ADCs can leverage the safety findings from patients treated with other ADCs that employ the same cytotoxic payload. These agents have the potential to offer a distinct and more benign safety profile relative to non-targeted microtubule inhibitors in men with advanced prostate cancer.

9. CLINICAL ANTITUMOR RESPONSES

9.1. Assessments of antitumor activity

Measurement of tumor response to therapy is challenging in prostate cancer (126). Bone represents a major site of metastasis; however, bony lesions are difficult to quantitate using conventional imaging methods. In addition, there is limited standardization of methods for imaging bone disease. As a result, most patients lack measurable lesions that can be assessed objectively for change. Given this situation, disease markers are used widely to assess progression and response to therapy.

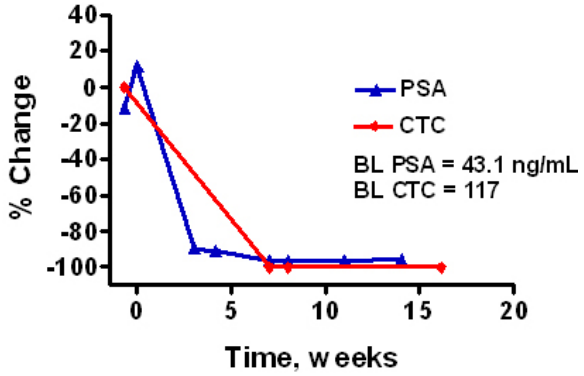

Figure 4. PSA and CTC responses in a subject treated with 2.0 mg/kg PSMA ADC in the 1301 study. CTCs are expressed as the number of cells per 7.5 mL of blood. BL = baseline.

Chief amongst these markers are serum PSA and circulating tumor cells (CTCs). PSA is used to initially screen individuals for prostate cancer, and prostate cancer patients and their physicians often closely monitor their PSA values for evidence of disease progression. PSA reductions of 50 percent or greater are often considered based on early associations between declines of this magnitude and overall survival (127); however, this endpoint has not been established as a surrogate for overall survival. In fact, patients may derive clinical benefit from PSA stabilizations and reductions of any magnitude, and thus PSA reductions of other magnitudes also are often reported in clinical studies.

CTCs represent rare cells that have been shed from a tumor mass and have entered the bloodstream (128). CTCs are an essential stage of tumor dissemination and formation of distant metastases. Many methods have been developed for detecting, counting, and isolating CTCs. Veridex (Warren, NJ) has established the CellSearch™ platform for enumerating CTCs, and this assay system presently is the only one cleared by FDA for assessing patient prognosis and treatment effectiveness (129). CTCs above a threshold level (5 CTCs per 7.5 mL of blood) represent an independent predictor of disease progression and overall survival in patients initiating a new line of therapy in prostate, breast, and colon cancers (130-133). CTCs are not currently an established surrogate of response to therapy in any disease; however, efforts are underway to potentially validate CTCs as a response indicator in prostate cancer (134). For these reasons, post-treatment changes in PSA and CTCs are commonly assessed in clinical studies of investigational agents in prostate cancer.

9.2. PSMA ADC

Although PSMA ADC was designed primarily as a first-in-humans safety study, changes from baseline in serum PSA and the numbers of CTCs were assessed. Assessments of measurable disease were not an important focus or performed at standardized intervals, and few subjects had measurable disease at baseline. Treatment of subjects in the core study concluded recently. Only preliminary efficacy findings are available as the database has not been locked as of this writing.

Antitumor activity as reflected in PSA and/or CTC responses was observed at 1.6 mg/kg and all higher doses. PSA reductions ranging to 90 percent and greater were observed in several subjects and often were observed to occur after the first dose of PSMA ADC. In other cases, PSA declined progressively with successive doses or stabilized initially and then declined following repeat dosing. PSA responses of greater than or equal to 50 percent typically were sustained throughout the 12-week core study. Reductions in both PSA and CTC were often observed in a given subject, as illustrated in Figure 4 for a subject treated with 2.0 mg/kg PSMA ADC. This subject experienced a 96 percent reduction in serum PSA and a complete loss of CTCs from an initial value of 117 cells per 7.5 mL of blood. The responses were sustained throughout the 12-week duration of the core study. The durable and coordinated nature of such responses serves to corroborate the activity signal seen for PSMA ADC. Similarly, CTC responses were observed in the setting of stable PSA, a finding that is further suggestive of clinical benefit. A complete description of these findings as well as final PSA and CTC response rates is planned for a future publication.

The preliminary efficacy findings indicate that PSMA ADC has definite anti-tumor activity in this heavily treated patient population over a range of tolerated doses. PSMA ADC was recently advanced into phase 2 testing in metastatic CRPC patients.

9.3. MLN2704

Efficacy assessments included post-therapy changes in serum PSA and in tumor burden as determined by computed tomography (CT) or magnetic resonance imaging (MRI) of subjects with measurable disease at baseline. PSA responses were defined as confirmed PSA decreases of greater than or equal to 50 percent from baseline. In addition, 25 percent reductions in PSA were reported in some studies. Changes in measurable tumor burden were evaluated by REsponse Criteria In Solid Tumors (RECIST).

For the twelve subjects treated with weekly MLN2704, no greater than or equal to 50 percent PSA responses were observed. However, a greater than or equal to 25 percent reduction was reported for one subject in the second of the four ascending dose levels. The effects of treatment of measurable disease were not reported.

Three greater than or equal to 50 percent PSA responses were reported across the 15 subjects treated with the Q2W regimen. At the highest dose (330 mg/m^2), two of six subjects had greater than or equal to 50 percent reductions in PSA, and two additional subjects had 25-50 percent reductions. One subject with a baseline PSA of 14 ng/mL experienced a 70 percent PSA decline that was sustained for approximately 5 months. This subject received two 330 mg/m^2 doses initially, followed by seven cycles at a reduced dose of 236 mg/m^2. No tumor regressions were reported for the Q2W regimen; however,

there were four reports of stable disease amongst the six subjects with measurable disease at baseline.

There were no greater than or equal to 50 percent PSA responses in the 18 subjects treated according to a Q3W schedule. Two greater than or equal to 25 percent PSA reductions were observed, one at each of the two dose levels tested, namely 330 mg/m^2 and 462 mg/m^2. Nine subjects had measurable disease at baseline. The best response was stable disease in four subjects.

The Q4W regimen showed evidence of antitumor activity in two subjects. One of the three subjects treated with 264 mg/m^2 experienced an approximately 80 percent reduction in PSA, an improvement in skin lesions, and a partial confirmed response by RECIST. This subject was taxane-naïve, had a baseline PSA of approximately 75 ng/mL and received 14 doses of study drug before discontinuing treatment at week 47 due to disease progression and grade 2 peripheral neuropathy. A PSA response was also observed in a subject who had received prior treatment with docetaxel. This subject experienced a DLT after an initial MLN2704 dose of 343 mg/m^2, but then reinitiated treatment at week 12 and received four doses of 264 mg/m^2. PSA levels declined 53 percent from a baseline of 44.8 ng/mL over approximately 100 days.

Of the seventeen subjects treated with 330 mg/m^2 according to a Q6W cycle, three experienced greater than or equal to 50 percent PSA responses and five had 25-50 percent declines. No information was provided on the durability of the PSA responses. Five subjects had measurable disease at baseline. The best response was stable disease in two subjects.

9.4. Lessons from clinical assessments of antitumor activity

PSMA-targeted ADCs appear to be clinically active in advanced prostate cancer. Treatment has been associated with potent and durable PSA responses that often are accompanied by other measures of antitumor activity. PSA responses are often rapid, as reflected in reductions ranging to 90 percent even after the first dose. In addition, a broader group of subjects have experienced apparent clinical benefit in the form of long-lived disease stabilization. The findings provide initial clinical validation of PSMA as a target for ADCs that incorporate microtubule inhibitors. Nevertheless, a group of subjects progressed through treatment without apparent benefit, and the observed variation in responses provides impetus for biomarker studies that may provide a means to enrich for patients who are good candidates for therapy.

10. BIOMARKERS

Other than the use of PSA and CTCs as potential markers of response to therapy, biomarker evaluations were not employed in the completed studies of PSMA ADC or MLN2704. Rather, both ADCs were tested in all-comer patient populations. As noted above, subjects exhibited variable responses to treatment with either PSMA ADC or MLN2704, with some subjects experiencing robust, durable responses while other subjects progressed through therapy. Therefore, opportunity exists to use biomarkers to prospectively identify and enrich for responder patients in future clinical studies of PSMA-targeted ADCs.

One biomarker of interest is PSMA expression. The selective activity of ADCs critically depends on the expression of the target antigen by the tumor, and target expression above a minimum threshold is required for optimal activity. Although PSMA is expressed in nearly all cases of prostate cancer (see Section 3), expression can vary between patients. Indeed, and as noted above, the variation in expression is both quantifiable and biologically significant in that PSMA expression provides an independent predictor of disease progression following primary intervention. Other studies suggest that PSMA expression may become more heterogeneous in advanced, metastatic disease. Variation has been reported both in terms of the intensity of expression per cell and in the percentage of cells within a given lesion that express the antigen. To this end, it is notable that both PSMA ADC and MLN2704 utilize cleavable linkers that release free drug with the capability for bystander cell killing (135, 136).

In addition, while PSMA is expressed in essentially all cases of prostate adenocarcinoma, it is not typically expressed in neuroendocrine prostate cancers (NEPC). NEPC is a histologically and phenotypically distinct subtype of prostate cancer that does not generally express AR, PSA or PSMA. NEPC is uncommon at diagnosis, representing 1 percent or less of prostate cancers (137). However, neuroendocrine transdifferentiation is a more frequent event that may be induced by androgen-deprivation therapy for prostate adenocarcinoma (138, 139). Autopsy studies indicate that a sizeable percentage of patients with prostate cancer die from NEPC-predominant disease, and the clinical significance of NEPC may increase following the recent introduction of highly potent antiandrogens (140). In addition to being distinguishable by histological evaluations, NEPC secrete markers such as chromogranin A and neuron-specific enolase, and serum levels of these markers have been associated with the extent of neuroendocrine differentiation in small studies (141-143). These latter markers thus may be of use in identifying patients who are poor candidates for therapy with PSMA-targeted agents. Pre-treatment data on PSMA expression and markers of NEPC are being collected in the PSMA ADC phase 2 program.

11. CONCLUSIONS

PSMA is a well-characterized and clinically validated cell-surface marker of prostate cancer, and it has many of the biological properties desired in a target for ADC therapy of cancer. Two PSMA-targeted ADCs have completed early-stage studies in men with advanced metastatic prostate cancer. In each case, treatment was associated with robust and durable PSA reductions and other measures of antitumor activity in a salvage group of heavily pre-treated patients with progressive disease. In addition to overt responses, prolonged stabilization of

disease has been observed in other patients. Antitumor activity was observed over a range of doses that were generally well tolerated, and the dose-limiting toxicities are those associated with release of free drug. The MTDs for anti-PSMA ADCs compare favorably with those of other ADCs that contain the same cytotoxic agent. Notably, no target-related toxicity has been identified that can be attributed to PSMA expression in any normal organ or tissue. The findings provide initial clinical proof of concept for ADC targeting of PSMA in prostate cancer, and phase 2 clinical testing of one agent is underway. This progress has opened the door to testing anti-PSMA ADCs as a novel antineovascular therapy of the broader range of solid tumors that express PSMA within the nascent tumor blood vessels. Finally, studies are ongoing to assess whether tumor expression of PSMA may provide a biomarker useful in prospectively identifying patients likely to respond favorably to treatment with ADCs against PSMA.

12. ACKNOWLEDGMENTS

The vcMMAE drug-linker was obtained under a license to Progenics from Seattle Genetics. We thank Patty Schneider for excellent graphical assistance and proofreading of the manuscript.

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Abbreviations: ADAM17: a disintegrin and metalloprotease domain family member 17, ADC: antibody-drug conjugate, ALT: alanine aminotransferase, AR: androgen receptor, AST : aspartate aminotransferase, CRPC: castration-resistant prostate cancer, CT: computed tomography, CTCs: circulating tumor cells, DLT: dose-limiting toxicity, DM1: maytansinoid 1, DM1-SH: free DM1, ELISA: enzyme-linked immunosorbent assay, GCP II: glutamate carboxypeptidase II, G-CSF: granulocyte colony-stimulating factor, HRP: horseradish peroxidase, LHRH: leutinizing hormone-releasing hormone, mAb: monoclonal antibody, mCRPC: metastatic CRPC, MMAE: monomethylauristatin E, MRI: magnetic resonance imaging, MTD: maximum tolerated dose, NAAG: N-acetlyaspartylglutamate, NAALADase: N-acetylated a-linked acidic dipeptidase, NEPC: neuroendocrine prostate cancer, PDS: 4-4'-dipyridyl disulfide, PK: : pharmacokinetic, PSA: prostate-specific antigen, PSCA: prostate stem-cell antigen, PSMA: prostate-specific membrane antigen, RECIST: REsponse Criteria In Solid Tumors, SPP: N-succinimidyl 4-(2-pyridyldithio)pentanoate, STEAP1 : six-transmembrane epithelial antigen of the prostate 1, TMEFF2: transmembrane protein with epidermal growth factor-like and two follistatin-like domains, vcMMAE: valine-citrulline monomethylauristatin E

Key Words: Prostate-specific membrane antigen; antibody-drug conjugate, PSMA ADC, MLN2704, Review

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