

# Abstract #724 A Phase 1 Clinical Trial of Immune Response and Safety of An Active Immunotherapy Regimen Co-targeting PRAME and PSMA Antigens in Subjects with Advanced Solid Malignancies\*

Mihail Obrocea<sup>1</sup>, Nicholas Vogelzang<sup>2</sup>, Lee Cranmer<sup>3</sup>, Marc S. Ernstoff<sup>4</sup>, Jeffrey Weber<sup>5</sup>, John Marshall<sup>6</sup>, Dar Rosario<sup>1</sup>, Zhiyong Qiu<sup>1</sup>, and Adrian Bot<sup>1</sup>  
 Author's affiliation: 1. MannKind Corp, Valencia, CA, 2. Nevada Cancer Institute, Las Vegas, NV, 3. Arizona Cancer Center, Tucson, AZ, 4. Dartmouth-Hitchcock Medical Center, Lebanon, NH, 5. Moffitt Cancer Center, Tampa, FL, 6. Lombardi Comprehensive Cancer Center, Georgetown, DC

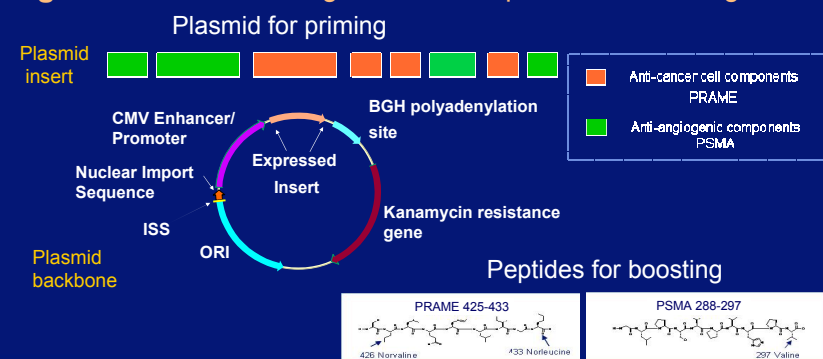
## Abstract

**Background:** This clinical trial is testing an investigational immunotherapeutic 3-component regimen encompassing a recombinant plasmid (pPRA-PSM) dose followed by 2 peptide (E-PSM and E-PRA) doses (low and high) delivered by intra lymph node injection in a prime-boost sequence aimed to elicit an immune response against PRAME and PSMA antigens. **Methods:** The trial design used a prime-boost strategy with all patients receiving a fixed priming dose (2,400 µg/dose) of the plasmid-antigen vector via ultrasound guided intranodal injection into inguinal or axillary lymph nodes on days 1, 4, 15 and 18 of each treatment cycle followed by peptide administrations on days 29 and 32. The peptide doses used are as follows: low dose cohort: E-PSM: 30 µg/dose, E-PRA: 22.5µg/dose; high dose cohort: E-PSM: 300µg/dose, E-PRA: 150µg/dose. All patients enrolled have advanced and/or refractory carcinomas, are HLA\*0201+ and tumor samples obtained from prior biopsies show presence of both PRAME and PSMA antigens. Immunologic evaluation occurs prior to treatment and on days 29 and 39 of each cycle with clinical evaluation for disease status every 2nd cycle. Patients that do not exhibit disease progression as defined by the protocol remain on study and may receive up to 6 cycles of treatment. Immune response as measured by flow cytometry using PBMCs quantifies the frequency of CD8+ tetramer+ T cells specific for PRAME and PSMA epitopes. In addition, a second immune response measurement, a direct ELISPOT analysis is used to determine the frequency of IFN gamma producing spot forming colonies. **Results:** Preliminary immune and clinical responses are available from 12 patients (6 melanoma, 4 prostate, 1 renal, 1 esophageal cancer and 1 mesothelioma) treated in the low peptide dose cohort. Ten patients completed 2 or more cycles of therapy. Positive immune responses (tetramer assay) against at least one immunizing antigen were noted in at least 6 patients with 3 patients showing sustained immune responses beyond 2 cycles of treatment. Most frequent treatment related adverse events reported were: fatigue, pain at injection site and chills (all grade 1 or 2). Clinical benefit was observed in 3 patients: 1 patient (ocular metastatic melanoma) completed 6 cycles with stable disease (SD), 1 patient (metastatic kidney cancer) had SD by end of cycle 4 and 1 patient (metastatic prostate cancer) had a decrease in the PSA > 60%. In the high dose peptide cohort, 10 patients received treatment with 6 patients on-going. **Conclusions:** To date treatment has been well-tolerated and positive immune responses were observed. A full clinical data set and any observed correlation(s) between immune responses and clinical outcome will be presented.

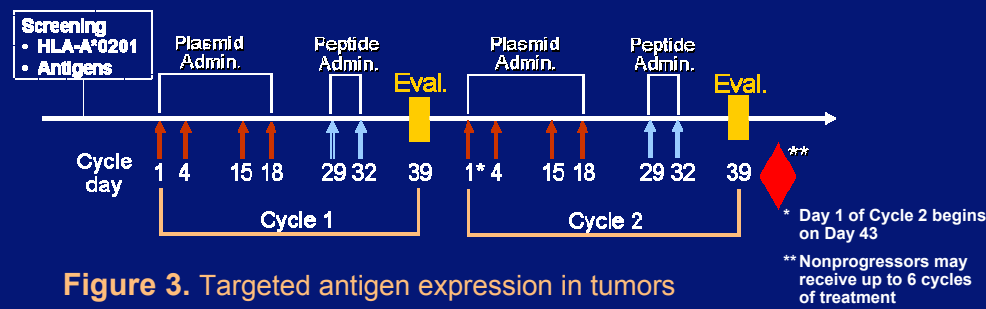
## Background and trial design

- Investigational drug
  - A recombinant plasmid expressing fragments of two targeted antigens, PRAME and PSMA (Fig.1), formulated as a sterile solution
  - 2 peptide analogues of PRAME 425-433 and PSMA 288-297 CTL epitopes (Fig.1), formulated as sterile solutions, respectively
- Drug administration
  - Injection: bolus injection directly into inguinal lymph nodes (Ref. 1-3)
  - Schedule: Plasmid DNA-prime, peptide-boost regimen (Fig.2, Ref.4)
- Trial objectives
  - Primary objective: immunologic response
  - Secondary objectives: to define the safety and adverse event profile, to assess plasmid persistency in the blood, and to describe objective response
- Eligibility
  - Patients with advanced solid malignancy refractory to standard chemotherapy, radiation, and/or surgery
  - HLA-A\*0201 positive and whose tumor express both PRAME and PSMA antigens

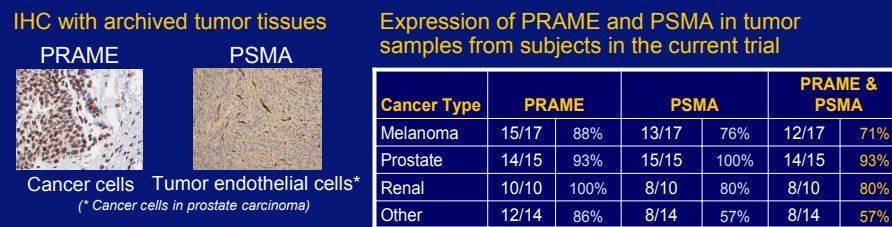
**Figure 1. Schematic diagram of active pharmaceutical ingredients**



**Figure 2. Treatment schedule**



**Figure 3. Targeted antigen expression in tumors**



## Immune monitoring results

**Table 1. Summary of preliminary tetramer response**

Subject number	Tumor type	Immunity at baseline		T cell expansion (cycles)		Total treatment cycles
		PRAME	PSMA	PRAME	PSMA	
Low dose cohort	L1 Prostate	-	-	3	3	4
	L2 Prostate	+	+	1	1	2
	L3 Prostate	+	+	0	0	2
	L4 Melanoma	+	+	0	1	2
	L5 Melanoma	+	+	0	0	2
	L6 Melanoma	+	-	6	6	6
	L7 Melanoma	+	+	0	0	2
	L8 Melanoma	+	+	0	0	2
	L9 Kidney	-	-	4	4	4
	L10 Esophageal	+	+	2	2	2
High dose cohort	H1 Prostate	+	+	0	0	3
	H2 Prostate	+	+	1	0	6
	H3 Prostate	-	-	3	3	4→
	H4 Prostate	-	+	1	0	2
	H5 Prostate	TBD	TBD	TBD	TBD	4→
	H6 Prostate	+	+	0	0	6
	H7 Prostate	-	-	0	1	3
	H8 Melanoma	+	+	0	0	2
	H9 Melanoma	-	-	1	1	2
	H10 Melanoma	-	-	1	1	2
	H11 Kidney	+	+	0	0	6

## Summary of preliminary ELISPOT results

- One ELISPOT+ responder from 21 subjects to date
- From high dose cohort, against PSMA
- No PRAME specific ELISPOT response detected to date

## Preliminary clinical observations

- Summary of preliminary clinical safety
  - No drug-related SAEs have been reported
  - Most frequent drug (probable, possible or certain) related AEs: Fatigue (intermittent) (Grade 1-2); unilateral and bilateral groin pain (Grade 1); fever (Grade 1); lightheadedness (Grade 1); rash, pruritis, flushing, erythema (Grade 1)

**Figure 4. Summary of preliminary clinical response in subjects who completed more than 2 cycles of treatment**

Subject number	Tumor type	Dose cohort	Wk 6	Wk 12	Wk 18	Wk 24	Wk 30	Wk 36
			Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
L3	Melanoma	Low						SD
L1	Prostate	Low		↓ PSA			PD	
H1	Prostate	High			PD			
H2	Prostate	High						SD
H3	Prostate	High		↓ PSA		→		
H5	Prostate	High		↓ PSA		→		
H7	Prostate	High			PD			
L9	Renal	Low					SD*	
H11	Renal	High						SD

\* Patient went for surgical resection of the metastasis and remains in CR (to date)

## Preliminary conclusions

- Expansion of T cells against PRAME and PSMA (measured in the peripheral blood) is observed in 60% of subjects in the trial after prime-boost immunization
- Data confirms that PRAME and PSMA antigens are widely expressed in a variety of different tumors with melanoma, prostate, and kidney cancers having the highest frequency
- Repetitive direct intra-lymph node administration is feasible in cancer patients, and the regimen is safe and well-tolerated
- Prior to immunization, 67% of subjects show presence of T cells against targeted PRAME and/or PSMA epitopes
- Clinical response is observed in 33% of subjects (low and high dose cohorts)

\* Trial in progress, all data preliminary

**References:** 1). Maloy, KJ., et al. (2001). *Proc. Natl. Acad. Sci. USA.* 98:3299-303. 2). Tagawa, ST., (2003). *Cancer* 98:144-54. 3). Weber, JS., (2008). *J. Immunother.* 31:215-23. 4). Smith, KA., et al. (2009). *Vaccine* 27: 2603-15.