

Samarium-153-EDTMP (Quadramet®) with or without vaccine in metastatic castration-resistant prostate cancer: A randomized phase 2 trial

Supplementary Materials

Supplementary Table S1: Adverse events, grade 3 and 4 only, all attributions

	Cohort A		Cohort B	
	Patients (% of total patients)	Events (events per dose Sm = 153)	Patients (% of total patients)	Events (events per dose Sm = 153)
Grade 3				
Hematologic	10 (45.5%)	22 (1.0)	13 (59.1%)	43 (1.1)
Anemia	7 (31.8%)	10 (0.4)	4 (18.2%)	6 (0.15)
ALC decreased	2 (9.1%)	2 (0.08)	3 (13.6%)	5 (0.13)
ANC decreased	2 (9.1%)	2 (0.08)	4 (18.2%)	9 (0.23)
Platelets decreased	3 (13.6%)	7 (0.28)	8 (36.4%)	13 (0.33)
WBC decreased	0 (0%)	1 (0.04)	5 (22.7%)	9 (0.23)
INR decreased	0 (0%)	0 (0)	1 (4.5%)	1 (0.03)
Gastrointestinal	3 (13.6%)	6 (0.24)	4 (18.2%)	6 (0.15)
Constitutional	3 (13.6%)	3 (0.12)	4 (18.2%)	5 (0.13)
Musculoskeletal	4 (18.2%)	3 (0.12)	5 (22.7%)	8 (0.21)
Electrolyte/Metabolic	1 (4.5%)	1 (0.04)	3 (13.6%)	3 (0.08)
Genitourinary	1 (4.5%)	1 (0.04)	0 (0%)	0 (0)
Infection	0 (0%)	0 (0)	1 (4.5%)	1 (0.03)
Dental	0 (0%)	0 (0)	1 (4.5%)	1 (0.03)
Grade 4				
Hematologic	2 (9.1%)	2 (0.08)	3 (13.6%)	3 (0.08)
Grade 5				
Death	1 (4.5%)	1 (0.04)	0 (0%)	0 (0)

Supplementary Table S2: Flow-cytometry analysis of PBMC immune subsets: 25 markers, 110 subsets

<p>1. CD4: Helper T lymphocytes (28 subsets)</p> <p>2. CD8: Cytotoxic T lymphocytes (25 subsets)</p> <p>Maturation status of T lymphocytes (in CD4 and CD8): Naïve: CD45RA⁺ CCR7⁺ Central Memory: CD45RA⁻ CCR7⁺ Effector Memory: CD45RA⁻ CCR7⁻ Terminal (EMRA): CD45RA⁺ CCR7⁻</p> <p>T lymphocyte markers (in CD4 and CD8): CTLA-4: inhibition PD-1: activation/inhibition PD-L1: activation/cross-inhibition TIM-3: inhibition ICOS: activation (only on CD4)</p> <p>3. Tregs: Regulatory T lymphocytes (CD4⁺ CD25⁺ FoxP3⁺ CD127⁻) (6 subsets) CD45RA: Tregs highly expandable <i>in vitro</i> CTLA-4: Treg suppression ICOS: Treg suppression PD-1: activation/inhibition PD-L1: cross-inhibition</p> <p>4. B lymphocytes: CD19⁺ (3 subsets) PD-1: activation/inhibition PD-L1: cross-inhibition</p>	<p>5. NK: Natural killer cells (CD56⁺ CD3⁻) (20 subsets) CD16⁺ CD56^{br}: Functional intermediate, lytic and cytokine production CD16⁺ CD56^{dim}: Mature NK, cytokine production CD16⁻ CD56^{br}: Immature, abundant in human placenta CD16⁻ CD56^{dim}: non-lytic, non-cytokine production TIM-3: activation PD-1: activation/inhibition PD-L1: cross-inhibition</p> <p>6. NK-T: CD56⁺ CD3⁺ (4 subsets) TIM-3: activation PD-1: activation/inhibition PD-L1: cross-inhibition</p> <p>7. cDCs (conventional DCs): CD3⁻ CD56⁻ CD11c^{br} CD303⁻ CD141⁻ (4 subsets)</p> <p>8. pDCs (plasmacytoid DCs): CD3⁻ CD56⁻ CD1c⁺ CD303⁺ (4 subsets) Markers of DC activation TIM-3: inhibition PD-1: activation/inhibition PD-L1: cross-inhibition</p> <p>9. MDSCs: Myeloid-derived suppressor cells (CD11b⁺ HLA-DR^{low} CD33⁺) (16 subsets) CD14: Common Myeloid Marker (high in monocytes, dim in granulocytes) CD15: Granulocyte marker CD68: Monocyte/macrophagemarker PD-1: activation/inhibition PD-L1: cross-inhibition</p>
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Subsets analyzed: 9 standard immune cell subsets, PD-L1 and PD-1 in standard subsets, and 83 additional subsets relating to maturation/function.

Supplementary Table S3: Standard immune cell subsets

Subset (% of PBMC)	Interquartile range of pre	Sm-153 Alone (n = 7)				Sm-153 + PROSTVAC (n = 9)			
		Median		P value (unadjusted)	P value (adjusted)	Median		P value (unadjusted)	P value (adjusted)
		Pre	Post			Pre	Post		
CD4	24.2–31.2	27.5	29.1	> 0.999	> 0.999	27.6	33.8	0.2031	> 0.999
CD8	15.2–25.1	23.2	29.0	0.0781	0.702	20.4	18.1	0.6523	> 0.999
Treg	2.02–3.11	2.53	2.73	0.3750	> 0.999	2.41	2.54	0.5703	> 0.999
NK	7.6–12.4	8.5	8.6	0.1563	> 0.999	11.1	8.8	0.8203	> 0.999
NK=T	0.61–1.58	1.141	1.178	0.5781	> 0.999	0.779	1.039	0.6523	> 0.999
B Lymphocyte	2.54–6.18	4.00	3.74	0.8125	> 0.999	5.04	4.96	0.6523	> 0.999
cDC	0.047–0.342	0.124	0.154	0.2969	> 0.999	0.128	0.096	0.8203	> 0.999
pDC	0.174–0.407	0.270	0.201	0.2188	> 0.999	0.198	0.259	> 0.999	> 0.999
MDSC	3.26–9.94	5.26	7.44	0.2969	> 0.999	4.60	8.70	0.2031	> 0.999

Abbreviations: Tregs, regulatory T cells; NK, natural killer cells; NK-T, natural killer T cells; cDC, conventional dendritic cells; pDC, plasmacytoid dendritic cells; MDSCs, myeloid-derived suppressor cells.

Supplementary Table S4: Immune cell subsets relating to maturation/function

Subset (% of PBMC)	Interquartile range of pre	Sm-153 Alone (<i>n</i> = 7)				Sm-153 + PROSTVAC (<i>n</i> = 9)			
		Median		<i>P</i> value (unadjusted)	<i>P</i> value (adjusted)	Median		<i>P</i> value (unadjusted)	<i>P</i> value (adjusted)
		Pre	Post			Pre	Post		
CD4 ICOS ⁺ PDL1 ⁺	0.129–0.246	0.166	0.155	0.0469 ⁺	> 0.999	0.204	0.124	0.0195	0.468
CD4 CM	0.395–1.1202	0.440	0.636	0.0469 ⁺	> 0.999	0.769	1.343	0.0195	0.468
CD8 PDL1 ⁺	0.176–0.305	0.207	0.266	0.5781	> 0.999	0.220	0.170	0.0391	0.897
CD8 PDL1 ⁺ EM	0.083–0.186	0.097	0.117	0.8125	> 0.999	0.129	0.072	0.0391	0.897
NK PD1 ⁺ CD16-CD56 ^{dim}	0.040–0.090	0.052	0.121	0.0156	0.288	0.067	0.070	0.4258	> 0.999
MDS C PDL1 ⁺	0.503–2.021	1.469	2.665	0.0156	0.224	0.534	0.891	0.0977	0.999
mMDS C PDL1 ⁺	0.266–1.107	0.421	2.063	0.0156	0.224	0.342	0.592	0.0195 ⁺	0.273
mMDS C PD1 ⁺	0.029–0.151	0.077	0.173	0.0156	0.224	0.054	0.136	0.0742	> 0.999

⁺Not considered a potentially biologically relevant change, because median post value falls within the interquartile range of pre. Bold indicates significant *P* values.

Supplementary Table S5: Antibodies for assessing frequency of peripheral immune cell subsets

Marker	Conjugate	Clone	Company
CTLA4	FITC	L3D10	BioLegend
PD1	PE	MIH4	BD Bioscience
CCR7	PerCp-Cy5.5	150503	BD Bioscience
PDL1	PE-Cy7	MIH1	BD Bioscience
Tim3	BV421	F38-2E2	BioLegend
CD4	BV605	RPA-T4	BD Bioscience
CD8	APC	RPA-T8	BioLegend
CD45RA	AF700	HI100	BD Bioscience
CD19	APC-H7	SJ25C1	BD Bioscience
ICOS	PerCP-Cy5.5	C398.4A	BioLegend
FoxP3	Pac. Blue	206D	BioLegend
CD25	APC	M-A251	BioLegend
CD127	APC-eF780	eBioRDR5	eBioscience
CD15	FITC	HI98	eBioscience
CD68	PerCp-Cy5.5	Y1/82A	BioLegend
CD14	V450	M0Pg	BD Bioscience
HLA-DR	BV605	L243	BioLegend
CD33	APC	WM53	BioLegend
CD11b	APC-Cy7	ICRF44	BD Bioscience
CD16	FITC	3G8	BD Bioscience
CD303	PerCp-Cy5.5	201A	BioLegend
CD11c	BV605	3.9	BioLegend
CD56	APC	MEM-188	BioLegend
CD141	BV711 (AF700)	1A4	BD Bioscience
CD3	APC Cy7	UCHT1	BioLegend

Supplementary Table S6: Antibodies for assessing antigen-specific responses

Marker	Conjugate	Clone	Company
TNF	PE	MAb11	BD Bioscience
CD4	PerCp-Cy5.5	OKT4	BioLegend
IFN γ	PE-Cy7	4S.B3	BD Bioscience
IL-2	BV521	MQ1-17H12	BioLegend
CD107a	APC	H4A3	BD Bioscience
CD8	AF700	RPA-T8	BD Bioscience