

## Supplementary Online Content

Heery CR, Ibrahim NK, Arlen PM, et al. Docetaxel alone or in combination with a therapeutic cancer vaccine (PANVAC) in patients with metastatic breast cancer: a randomized clinical trial. *JAMA Oncol*. Published online August 20, 2015. doi:10.1001/jamaoncol.2015.2736.

**eTable.** Immune responses developed to the tumor associated antigens (TAAs) CEA or MUC-1 (encoded in the vaccine), or the cascade antigen brachyury following docetaxel +/- PANVAC therapy.

**eFigure.** Representative histograms from patient #22 pre and post treatment with docetaxel + PANVAC showing the development of antigen specific responses to CEA and MUC-1 following therapy.

**eTable. Immune responses developed to the tumor associated antigens (TAAs) CEA or MUC-1 (encoded in the vaccine), or the cascade antigen brachyury following docetaxel +/- PANVAC therapy.**

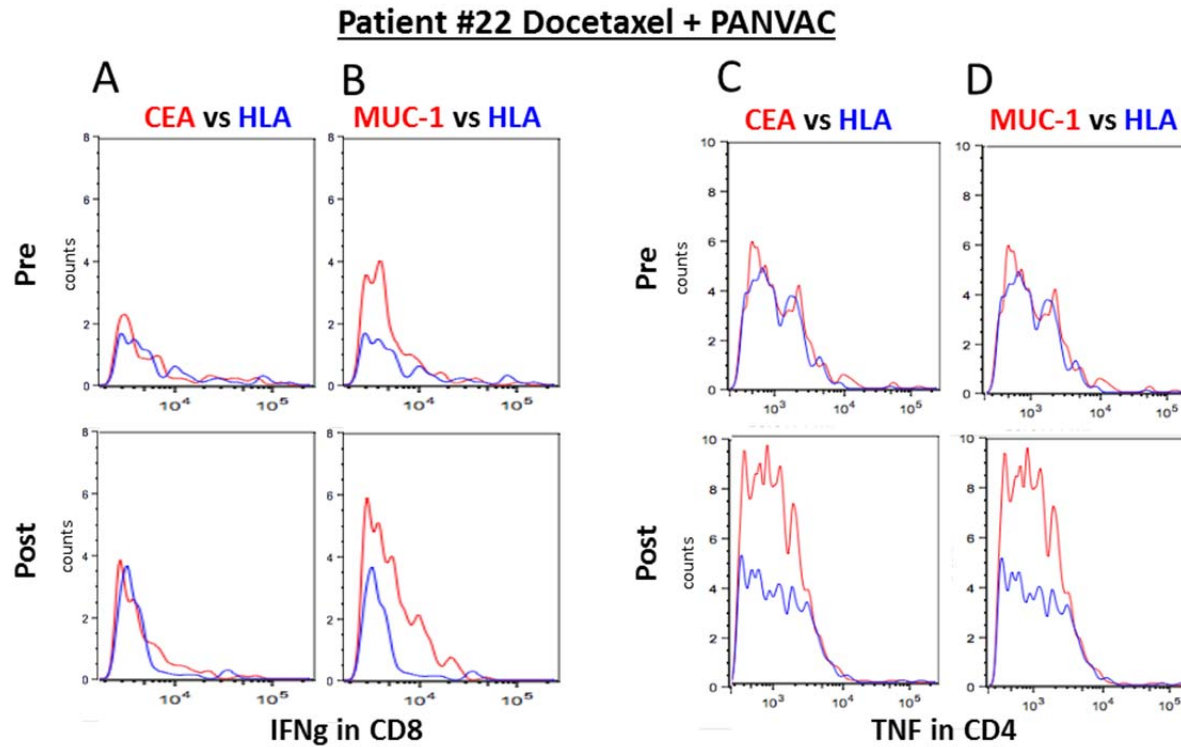
Docetaxel + PANVAC	CD4 T lymphocytes									CD8 T lymphocytes									Number of Responses to CEA/MUC-1 /Brachyury	Number of Responses to CEA /MUC-1
	CEA			MUC-1			Brachyury			CEA			MUC-1			Brachyury				
	IFNg	IL2	TNF	IFNg	IL2	TNF	IFNg	IL2	TNF	IFNg	IL2	TNF	IFNg	IL2	TNF	IFNg	IL2	TNF		
PT #6	0	0	0	0	0	0	np	np	np	0	38	<b>454</b>	0	42	0	np	np	np	11/16 (69%)	9/16 (56%)
PT #7	0	0	76	0	0	148	np	np	np	0	0	0	0	0	0	np	np	np		
PT #9	0	0	0	165	0	0	np	np	np	0	0	0	226	0	0	np	np	np		
PT #10	0	0	0	<b>276</b>	<b>409</b>	79	191	0	0	0	0	0	109	58	0	0	0	0		
PT #14	74	0	158	62	81	<b>460</b>	np	np	np	158	0	0	111	0	111	np	np	np		
PT #16	186	<b>466</b>	<b>406</b>	16	<b>514</b>	<b>725</b>	0	<b>444</b>	0	0	54	0	0	2	<b>810</b>	0	0	0		
PT #19	0	0	155	0	31	<b>347</b>	np	np	np	0	117	33	0	<b>275</b>	127	np	np	np		
PT #22	122	0	<b>736</b>	<b>1495</b>	0	<b>2650</b>	0	0	0	0	0	169	<b>2842</b>	0	<b>9587</b>	0	0	0		
PT #23	0	220	82	0	69	0	0	109	0	0	31	0	0	92	0	0	0	<b>300</b>		
PT #25	0	<b>300</b>	154	0	0	0	95	0	0	0	0	0	0	165	0	61	0	0		
PT #201	0	0	0	0	190	<b>331</b>	np	np	np	0	0	0	0	8	0	np	np	np		
PT #203	0	<b>436</b>	<b>370</b>	0	0	0	0	0	0	0	30	0	0	94	0	0	0	0		
PT #207	0	198	105	0	62	28	0	203	<b>313</b>	12	24	27	0	66	104	0	4	77		
PT #210	0	0	0	0	0	0	0	0	0	0	18	0	0	0	0	0	62	0		
PT #214	0	0	0	0	0	0	np	np	np	0	0	0	0	41	0	np	np	np		
PT #216	0	0	0	131	0	0	np	np	np	0	0	0	0	0	0	np	np	np		

Docetaxel Alone	CD4 T lymphocytes									CD8 T lymphocytes									Number of Responses to CEA/MUC-1 /Brachyury	Number of Responses to CEA /MUC-1
	CEA			MUC-1			Brachyury			CEA			MUC-1			Brachyury				
	IFNg	IL2	TNF	IFNg	IL2	TNF	IFNg	IL2	TNF	IFNg	IL2	TNF	IFNg	IL2	TNF	IFNg	IL2	TNF		
PT #5	0	0	0	0	0	0	np	np	np	0	0	0	0	0	0	np	np	np	8/15 (53%)	6/15 (40%)
PT #8	0	0	0	0	0	0	0	0	0	<b>414</b>	0	<b>2330</b>	0	0	0	0	56	0		
PT #11	<b>330</b>	0	0	<b>269</b>	0	<b>259</b>	0	0	0	0	0	<b>1154</b>	<b>960</b>	14	<b>4392</b>	0	0	0		
PT #12	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PT #13	0	0	0	0	0	0	0	0	0	39	0	0	26	0	0	0	0	0		
PT #15	0	21	239	0	0	32	<b>352</b>	134	138	0	0	0	0	0	0	<b>386</b>	58	<b>1466</b>		
PT #17	0	0	0	0	0	0	np	np	np	0	0	0	0	0	0	np	np	np		
PT #20	132	0	0	0	0	0	55	0	60	0	94	0	0	105	0	0	95	0		
PT #21	0	0	0	199	1	0	139	1	0	0	118	0	11	0	0	0	0	0		
PT #24	0	<b>350</b>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
PT #200	<b>353</b>	0	0	241	0	0	77	0	0	129	0	0	84	0	38	87	10	170		
PT #206	146	0	0	61	0	0	0	0	0	59	0	0	202	90	207	8	0	0		
PT #208	<b>692</b>	0	0	<b>329</b>	0	0	<b>356</b>	0	0	<b>405</b>	0	0	<b>278</b>	25	0	0	0	0		
PT #212	0	0	0	0	0	0	0	212	<b>572</b>	0	0	0	0	0	0	0	228	76		
PT #217	235	0	0	<b>261</b>	<b>862</b>	<b>1944</b>	176	0	<b>310</b>	<b>565</b>	23	0	<b>401</b>	<b>1054</b>	<b>1318</b>	0	0	0		

Values in the table represent the absolute number post- minus pre-therapy of CD4<sup>+</sup> or CD8<sup>+</sup> T lymphocytes, per 1x10<sup>6</sup> cells plated at the start of the in vitro stimulation (IVS), that produced IFN-γ, IL2, or TNF in response to stimulation with TAA 15-mer peptide pools in IVS assay. These values were generated by first subtracting background values (i.e., any signal obtained from the IVS with the negative control peptide pool HLA) from that obtained with CEA, MUC-1 or brachyury 15-mer peptide pools, and then subtracting pre-therapy values from post-therapy values [(Post TAA – Post HLA) – (Pre TAA – Pre HLA)]. An antigen-specific immune response to a given tumor-associated antigen was scored as positive (seen in bold type) if a patient had >250 CD4<sup>+</sup> or CD8<sup>+</sup> T

lymphocytes that produced IFN- $\gamma$ , IL2, or TNF at the end of the IVS per  $1 \times 10^6$  cells plated at the start of the IVS (following subtraction of any background and pre-therapy signal). Bold numbers indicate patients who met the immune response criteria defined above.

**eFigure. Representative histograms from patient #22 pre and post treatment with docetaxel + PANVAC showing the development of antigen specific responses to CEA and MUC-1 following therapy.**



(A) IFN- $\gamma$  production by CD8<sup>+</sup> T lymphocytes following an in vitro stimulation (IVS) with 15-mer peptide pools encoding CEA or the negative control HLA pre- and post-therapy. (B) IFN- $\gamma$  production by CD8<sup>+</sup> T lymphocytes following IVS with 15-mer peptide pools encoding MUC-1 or HLA pre- and post-therapy. (C) TNF production by CD4<sup>+</sup> T lymphocytes following IVS with 15-mer peptide pools encoding CEA or HLA pre- and post-therapy. (D) TNF production by CD4<sup>+</sup> T lymphocytes following IVS with 15-mer peptide pools encoding MUC-1 or HLA pre- and post-therapy.