Supplementary Table 5. Relative frequency of T cell reactivities in the circulating or tumor-infiltrating T cell subsets targeting mutated neoantigens, self-antigens, and the autologous tumor cell lines

		Peripheral blood				Tumor	
		$CD8^+PD\text{-}1^+$		$CD8^+PD\text{-}1^{hi}$		$CD8^+PD\text{-}1^+$	
Patient ID		**Percentage 4-1BB$^+$**	**Percentage of total reactivities detected**	**Percentage 4-1BB$^+$**	**Percentage of total reactivities detected**	**Percentage 4-1BB$^+$**	**Percentage of total reactivities detected**
NCI-3998	$MAGEA6_{E168K}$ (TMG1)	2.4	10.0	2.9	8.8	3.8	30.1
	$PDS5A_{Y1000F;H1007Y}$ (TMG3)	0.6	2.5	0.5	1.5	0.2	1.6
	$MED13_{P1691S}$ (TMG5)	0.3	1.3	N.D.	N.D.	0.9	7.4
	Mutated antigens	**3.3**	**13.8**	**3.4**	**10.3**	**4.9**	**40.2**
	NY-ESO-1	20.7	86.2	29.7	89.7	7.3	59.8
	Self-antigens	**20.7**	**86.2**	**29.7**	**89.7**	**7.3**	**59.8**
	3998mel	9.5		7.2			11.2
NCI-3784	$FLNA_{R2049C}$ (TMG3)	0.4	16.0	1.4	26.4	0.4	16.0
	$KIB16B_{L1009P}$ (TMG5)	0.4	16.0	0.4	7.5	0.6	24.0
	SON_{R1927C} (TMG8)	1	40.0	0.6	11.3	0.6	24.0
	Mutated antigens	**1.8**	**72.0**	**2.4**	**45.3**	**1.6**	**64.0**
	MAGEA3	N.D.	**N.D.**	0.8	15.1	N.D.	N.D.
	GP100	0.7	28.0	2.1	39.6	0.9	36.0
	Self-antigens	**0.7**	**28.0**	**2.9**	**54.7**	**0.9**	**36.0**
	3784mel	*24.6		*44.9			*45.2
NCI-3903	$KIF1BP_{P246S}$ (TMG9)	1.3	39.4			5.8	75.3
	TMG4 (antigen unknown)	N.D.	N.D.			0.3	3.9
	Mutated antigens	**1.3**	**39.4**			**6.5**	**79.2**
	SSX2	2.0	60.6			1.6	20.8
	Self-antigens	**2.0**	**66.6**			**1.6**	**20.8**
	3903mel	7.8					10.2
NCI-3926	**Mutated antigens**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
	NY-ESO-1	1.7	100.0	1.4	100.0	3.4	70.8
	MART1	N.D.		N.D.		0.5	10.4
	GP100	N.D.		N.D.		0.9	18.8
	Self-antigens	**1.7**	**100.0**	**1.4**	**100.0**	**4.8**	**100.0**
	3926mel	*6.3		*7.7			*13.3

The absolute and relative frequency of circulating or tumor-infiltrating $CD8^+PD\text{-}1^+$ T cells targeting mutations, self-antigens, and the corresponding autologous tumor cell lines (3998mel, 3784mel, 3903mel, or 3926mel) is shown. Recognition of mutated antigens (TMGs), self-antigens (NY-ESO-1, MAGEA3, SSX2, MART1, GP100, and TYR), and the autologous tumor cell line for each of the patients was evaluated simultaneously in one experiment. IFN-γ ELISPOT and 4-1BB up-regulation were analyzed at 20 h. Only the frequencies from the conditions that demonstrated >40 spots and >twice the background by IFN-γ ELISPOT are shown. For subjects NCI-3998 and 3903 the frequency of T cells recognizing a given antigen was based on the percentage 4-1BB up-regulation at 20 h after coculture. Data are gated on $CD3^+$ cells. The frequency of 4-1BB expression against the irrelevant control was subtracted. For subjects NCI-3784 and 3926 the frequency of the reactivities was calculated based on IFN-γ ELISPOT data, as the frequencies were low and sometimes undetectable over background using 4-1BB upregulation. N.D. = Not detected. *The frequency of tumor-reactive cells for subjects NCI-3784 and NCI-3926 using IFN-γ ELISPOT was off-scale. For these, the frequency of autologous tumor-reactive cells was based on the percentage of 4-1BB up-regulation. Data are representative of at least two independent experiments