

## Online Resource 1

Peptides used for ELIspot assays.

### a) E6

<i>Sequence</i>	<i>Residues</i>
MHQKRTAMFQDPQER	1-15
AMFQDPQERPRKLPQ	7-21
QERPRKLPQLCTELQ	13-27
LPQLCTELQTTIHDI	19-33
ELQTTIHDIILECVY	25-39
HDIILECVYCKQQLL	31-45
CVYCKQQLLRREVYD	37-51
QLLRREVYDFAFRDL	43-57
VYDFAFRDLCIVYRD	49-63
RDLCIVYRDGNPYAV	55-69
YRDGNPYAVCDKCLK	61-75
YAVCDKCLKFYISKIS	67-81
CLKFYISKISEYRHYC	73-87
KISEYRHYCYSLYGT	79-93
HYCYSLYGTTLEQQY	85-99
YGTTLEQQYNKPLCD	91-105
QQYNKPLCDLLIRCI	97-111
LCDLLIRCINCQKPL	103-117
RCINCQKPLCPEEKQ	109-123
KPLCPEEKQRHLDKK	115-129
EKQRHLDKKQRFHNI	121-135
DKKQRFHNIIRGRWTG	127-141
HNIRGRWTGRCMSCC	133-147
WTGRCMSCCRSSRTR	139-153
SCCRSSRTRRETQL	145-158

### b) E7

<i>Sequence</i>	<i>Residues</i>
MHGDTPTLHEYMLDL	1-15
TLHEYMLDLQPETTD	7-21
LDLQPETTDLYCYEQ	13-27
TTDLYCYEQLNDSSE	19-33
YEQLNDSSEEEDEID	25-39
SSEEEDEIDGPAGQA	31-45
EIDGPAGQAEPDRAH	37-51
GQAEPDRAHYNIVTF	43-57
RAHYNIVTFCKCDS	49-63
VTFCKCDSTLRLCV	55-69
CDSTLRLCVQSTHVD	61-75
LCVQSTHVDIRTLED	67-81
HVDIRTLEDLLMGTL	73-87
LEDLLMGTLGIVCPI	79-93
GTLGIVCPICSQKP	85-98

## c) PPP

<i>Sequence</i>	<i>Pathogen and Protein</i>	<i>Residue</i>
PKYVKQNTLKLAT	Influenza A HA	306-324
QYIKANSKFIGITEL	TETANUS	830-844
FNNFTVSFWLRVPKVSASHLE	TETANUS	947-967
TSLYNLRRGTALA	EBNA1	515-527
AGLTLSLLVICSYLFISRG	EBV BHRF1	171-181
IVTDFSVIKAIEEEE	EBV EBNA3c	416-429
LTKGILGFVFTLTVPSERG	Flu M1	55-73
IQNAGLCTLVAMLEE	EBV lytic	276-290
RPFFHPVGEADYFEY	EBNA1	403-417
QEFFWDANDIYRIFA	HCMVpp65	511-525
QTEENLLDFVRFMGV	EBNA3c	281-290
RKTPRVTTGGGAMAGA	CMVpp65	415-429
RPQKRPSICGCKGT	EBNA1	71-85
RKCRAKFQLLQHYR	BZLF1	187-201
IQMCTELKLSDYEGR	Flu NP	41-55
SALILRGSVAHKSCLE	Flu NP	265-273
STLELRSRYWAIRTR	Flu NP	377-391
AAFEDLRVLSFIKGTK	Flu NP	336-351

## Online Resource 2. Summary of ELISPOT Results.

Patient	E6 Pre-therapy				E6 Post-therapy			
	Mean PBMC	SD	Mean PBMC+ E6 pep	SD	Mean PBMC	SD	Mean PBMC+ E6 pep	SD
2	18.0	2.8	<b>46</b>	15.4	27	11	<b>85</b>	25.3
3	90.7	4.8	<b>134</b>	6.2	34	1	<b>53</b>	16.6
4	102.0	11.9	94	13.5	78	17	44	11
5	154.0	4.1	<b>207</b>	5.2	111	13.5	105	8
6	39.0	9.9	61	7.4	65	25	52	6.5
7	10.0	1.0	<b>54</b>	13.8	27	12	<b>89</b>	11.8
8	122.3	1.7	129	10.2	88	9.4	85	8.1
9	45.0	17.5	<b>294</b>	21.1	31	5.1	<b>335</b>	24.1
16	79.0	3.6	<b>134</b>	4.9	17	1.7	27	1.2
17	25.0	4.2	<b>73</b>	13.7	15	3.4	31	5
18	14.3	3.8	<b>74</b>	7.4	5	1	8	2
19	38.0	4.0	<b>75</b>	5.6	35	2.5	31	0.5
20	107.7	5.6	<b>155</b>	6.6	67	11.4	95	6.2

Resp 10/13

% Resp 76.92

Resp 4/13

% Resp 30.77

Patient	E7 Pre-therapy				E7 Post-therapy			
	Mean PBMC	SD	Mean PBMC+ E7 pep	SD	Mean PBMC	SD	Mean PBMC+ E7 pep	SD
2	19	2.6	<b>256</b>	58	62	0.5	52	4.5
3	94	19.9	101	28.4	52	6.5	53	4.5
4	206	1.2	<b>285</b>	10.1	116	4.5	104	15.4
5	178	18.4	<b>241</b>	4.1	169	14.6	183	9.9
6	104	5.9	<b>168</b>	4.5	50	5	60	8.2
7	171	0.5	153	12.8	126	30.2	128	18.4
8	197	3.4	199	6	79	7.8	<b>138</b>	23.4
9	137	18.4	144	1.6	97	0.5	103	12.6
16	77	11.7	<b>119</b>	20.3	68	3.6	<b>75</b>	4.2
17	62	5	<b>113</b>	22.5	16	4	25	2.4
18	85	4.5	<b>160</b>	10.9	5	0	10	5
19	43	5	56	2	43	1	75	1.5
20	123	5.3	<b>177</b>	10.3	91	5.7	81	5.2

Resp 8/13

% Resp 61.54

Resp 2/13

% Resp 15.38

Patient	PPP Pre-therapy				PPP Post-therapy			
	Mean PBMC	SD	Mean PBMC+ PPP pep	SD	Mean PBMC	SD	Mean PBMC+ PPP pep	SD
2	176	34.5	<b>288</b>	11	74	5.5	<b>100</b>	13.7
3	43	9.9	<b>158</b>	16.4	59	4	<b>99</b>	12.3
4	191	21.6	239	11.5	159	1	<b>205</b>	20.2
5	173	3.4	<b>252</b>	9.3	138	10.2	129	8.3
6	180	24.6	237	4.3	49	0.5	61	5.4
7	155	2	<b>326</b>	13.3	55	8.5	<b>366</b>	5.2
8	196	11.3	<b>311</b>	14.6	68	8.2	<b>121</b>	10.6
9	136	13.1	<b>420</b>	9.4	110	10.9	<b>424</b>	21.6
16	149	13.9	<b>286</b>	15.5	65	6.1	<b>190</b>	2.2
17	156	7.8	<b>234</b>	8.1	57	7.8	<b>182</b>	31.6
18	48	7.3	<b>112</b>	15.9	6	1	<b>37</b>	13.7
19	70	12.5	<b>202</b>	2.9	55	0	<b>156</b>	10.5
20	54	7.6	<b>323</b>	9	154	10.5	<b>272</b>	33

Resp 11/13

% Resp 84.62

Resp 11/13

% Resp 84.62

Mean spot counts were determined by AID plate reader. Results in bold, signify positive responses according to the criteria described in Materials and Methods. Results in italics denotes use of duplicate rather than triplicate wells due to insufficient cell recovery from cultures.

### Online Resource 3. Representative ELISPOT Responses.

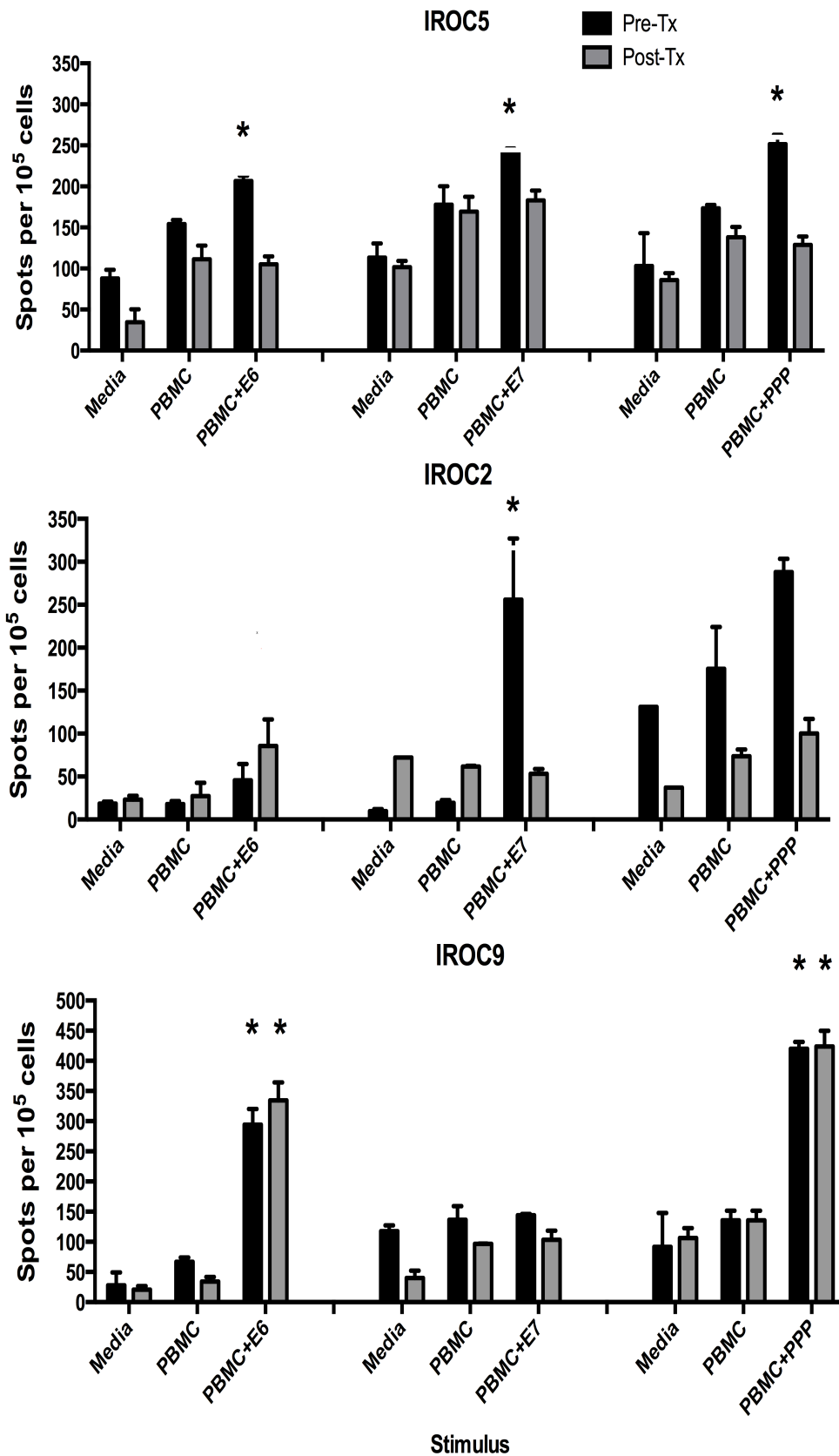


Fig S1. HPV16 peptide specific T cell responses in patients with oropharyngeal cancer. PBMC taken before (pre-Tx) and after therapy (post-Tx) were cultured with E6, E7 or PPP (recall) peptides then tested in IFN $\gamma$  ELISPOT assays against Media, PBMC, or PBMC+peptide. Representative graphs for patients with decreased responses post-therapy (IROC 5 and 2) or unchanged responses (IROC9). Graphs show mean spot counts. with \* denoting positive responses.

## Online Resource 4. Summary of Patient Details, Immune Cell Parameters and Clinical Follow-up

<i>Patient / Tumour details</i>				<i>Immune cell parameters</i>				<i>Treatment details</i>				<i>Followup status (August 2013)</i>						
<i>Study number</i>	<i>Pre-treatment TNM stage Site</i>		<i>HPV type</i>	<i>ELISPOT E6 preTx</i>	<i>ELISPOT E6 postTx</i>	<i>ELISPOT E7 preTx</i>	<i>ELISPOT E7 postTx</i>	<i>Increase Tregs</i>	<i>Increase MDSC</i>	<i>Neoadjuvant chemo</i>	<i>Definitive Platinum based CRT</i>	<i>RT + Concurrent Cetuximab</i>	<i>Primary Surgery Post-op RT</i>	<i>Progression</i>	<i>Disease free</i>	<i>Alive / Dead</i>	<i>Duration of follow up (months)</i>	<i>Time to progression (months)</i>
	IROC02	T2N2c																
IROC03	T2N2b	tongue base	HPV16	+	+	-	-	-	+	PF	0	1	0	0	1	A	17.7	
IROC04	T4aN2c	tongue base	HPV16	-	-	+	-	+	+	TPF	1	0	0	1 (lung)	0	A	23.8	21.0
IROC05	T4aN0	tongue base	HPV16	+	-	+	-	+	+	TPF	1	0	0	0	1	A	20.4	
IROC06	T2N2b	tonsil	HPV16	-	-	+	-	+	+	0	0	0	1	0	1	A	20.7	
IROC07	T4aN2a	tongue base	Not tested	+	+	-	-	+	-	0	1	0	0	0	1	A	10.5	
IROC08	T2N2b	tonsil	HPV16	-	-	-	+	+	+	0	0	0	1	0	1	A	15.2	
IROC09	T4bN2c	tonsil	HPV16	+	+	-	-	+	+	TPF	1	0	0	0	1	A	19.0	
IROC16	T2N2b	tonsil	no HPV	+	-	+	+	+	-	TPF	1	0	0	0	1	A	8.4	
IROC17	T2N2c	tonsil	no HPV	+	-	+	-	+	+	PF	0	1	0	1 (local)	0	D	5.7	4.8
IROC18	T4bN2c	tonsil	no HPV	+	-	+	-	+	+	TPF	1	0	0	0	1	A	12.9	
IROC19	T2N2b	tonsil	HPV16	+	-	-	-	+	+	TPF	0	1	0	0	1	A	10.1	
IROC20	T1N2b	tongue base	HPV16	+	-	+	-	+	+	0	1	0	0	0	1	A	15.1	

Study number (IROC#) corresponds to patient number in Results section

Elispot responses; +=significant response, -=no response

Increase in phenotypic markers; += increase, -= no increase, ND=not done, insufficient sample for phenotypic analysis

Abbreviations: TPF= chemotherapy with docetaxel (T), cisplatin (P) and 5 Fluorouracil (F), CRT = concurrent platinum based chemotherapy and radiotherapy