

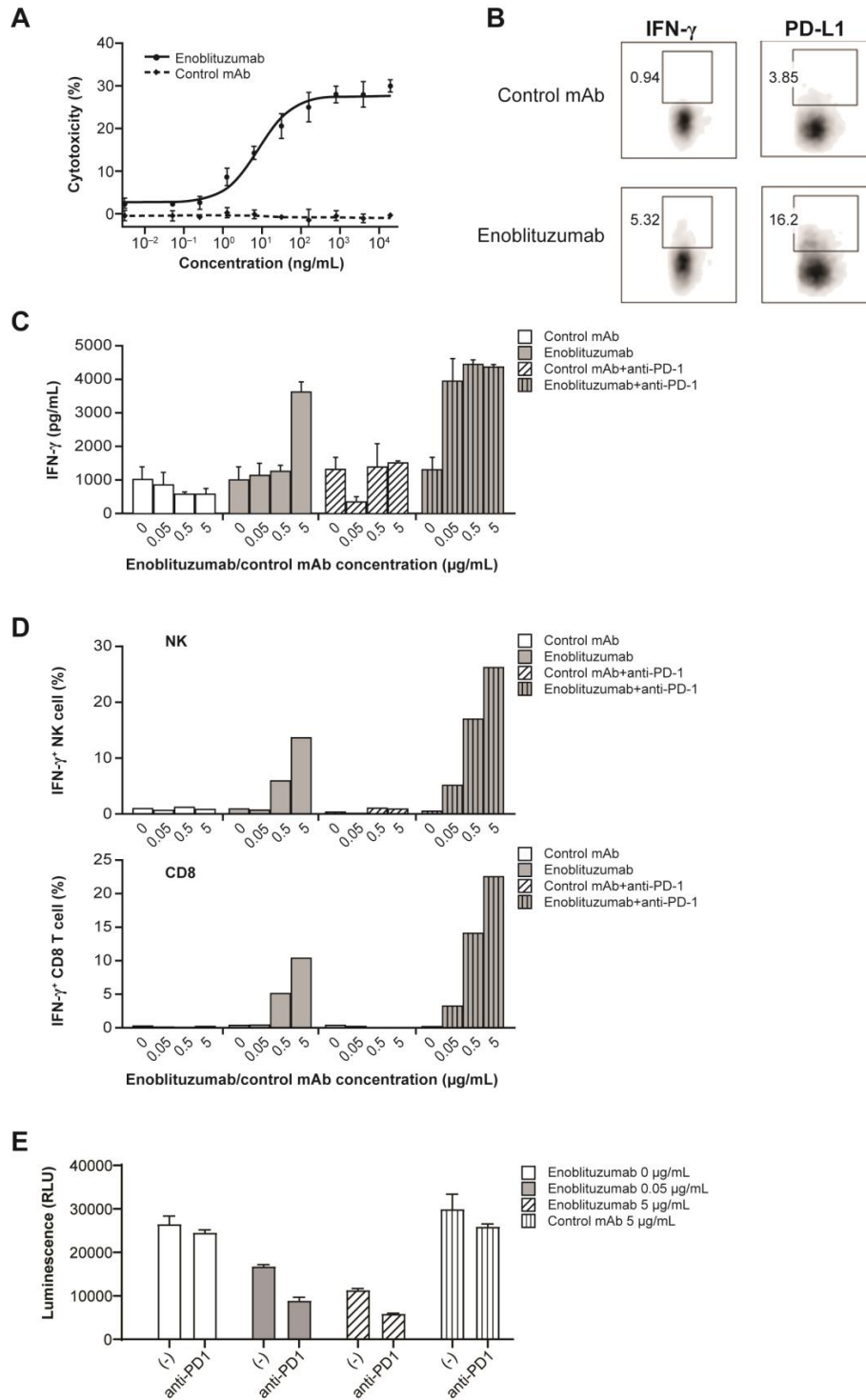
Journal for ImmunoTherapy of Cancer

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SUPPLEMENTAL MATERIAL

Dual checkpoint targeting of B7-H3 and PD-1 with enoblituzumab and pembrolizumab in advanced solid tumors: interim results from a multicenter phase I/II trial

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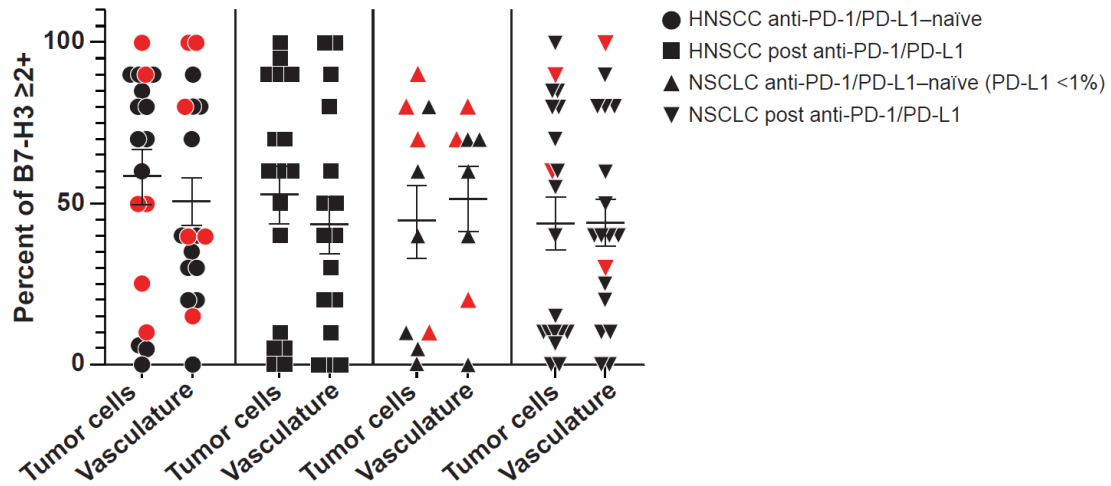


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Supplemental figure S1 Anti-PD-1 sustains enoblituzumab-mediated immune responses. (A)

Dose-dependent cytotoxicity targeting SAS (B7-H3–positive HNSCC) tumor cells mediated by enoblituzumab. Data shown as mean with SD. (B) Enoblituzumab mediated the upregulation of intracellular IFN- γ (measured at day 1) and surface PD-L1 (measured at day 2) on NK cells from PBMCs cocultured with SAS target cells. The values in FACS plots represent the percent of positive cells within the NK cell gate. (C) The levels of IFN- γ in supernatant from PBMCs cocultured with SAS after stimulation with enoblituzumab alone or in combination with anti-PD-1 mAb for 4 days. Data shown as mean with SD. (D, E) PBMCs were cocultured with SAS in the presence of enoblituzumab alone or in combination with anti-PD-1 mAb for 6 days. (D) Cells were collected and restimulated with PMA/ionomycin in the presence of Golgistop. Levels of NK cell and CD8 T cell specific intracellular IFN- γ was determine by FACS. (E) Alternatively, cells were evaluated for ADCC against the B7-H3 expressing tumor cell line (NCI H1975-luc) at an E:T ratio of 15:1. The loss of luminescence signal was used to measure the target cell lysis. Data shown as mean with SD. Data represent 1 of at least 3 (A, B, C, D) or 2 (E) different experiments using PBMCs from different donors.

Supplemental figure S2 B7-H3 $\geq 2+$ staining levels on tumor cells or vasculature.

Note: red symbols denote responders (CR and PR). B7-H3, B-cell antigen 7 homolog 3;

HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; PD-1,

programmed cell death-1; PD-L1, programmed death-ligand 1.

1 **Supplemental table S1** Biomarker status of patients with HNSCC or NSCLC

HNSCC, anti-PD-1/PD-L1-naïve																					
Patient number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18			
B7-H3	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+			
B7-H3 (tumor)	6	5	70	90	0	70	80	85	80	60	90	90	100	90	50	10	50	25			
B7-H3 (vasculature)	35	80	40	90	0	70	20	30	30	80	40	20	80	100	40	40	100	15			
PD-L1*	-	-	-	+	-	NA	+	-	+	-	+	NA	+	+	-	+	NA	+			
HNSCC, post anti-PD-1/PD-L1																					
Patient number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18			
B7-H3	+	+	+	-	+	NA	+	+	-	+	-	+	+	+	NA	+	+	+			
B7-H3 (tumor)	95	90	100	10	70	NA	60	90	5	40	5	0	60	90	0	70	60	50			
B7-H3 (vasculature)	100	20	100	20	10	NA	50	0	0	60	0	90	30	40	NA	50	40	80			
PD-L1*	+	+	+	+	+	NA	+	NA	-	NA	+	+	-	+	-	+	-	+			
NSCLC, anti-PD-1/PD-L1 with PD-L1 TPS <1%																					
Patient number	1	2	3	4	5	6	7	8	9	10	11	12	13	14							
B7-H3	+	-	NA	NA	-	+	NA	+	+	+	+	+	+	NA	+						
B7-H3 (tumor)	60	0	NA	NA	5	80	NA	40	10	90	10	80	NA	70							
B7-H3 (vasculature)	70	0	NA	NA	NA	40	NA	70	60	80	20	70	NA	NA							
PD-L1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
NSCLC, post anti-PD-1/PD-L1																					
Patient number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21

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B7-H3	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	NA	+	+	+	+
B7-H3 (tumor)	10	7	85	80	0	10	70	10	15	10	100	10	40	85	0	80	NA	60	55	90	60	
B7-H3 (vasculature)	80	10	50	80	25	90	0	10	80	NA	40	20	60	40	40	NA	NA	40	0	100	30	
PD-L1*	-	+	NA	-	NA	+	-	-	-	+	+	-	-	-	+	NA	-	+	-	+	+	

- 2 *PD-L1 positivity defined as TPS \geq 1%. B7-H3, B-cell antigen 7 homolog 3; HNSCC, head and neck squamous cell carcinoma; NA,
- 3 not available; NSCLC, non-small cell lung cancer; PD-1, programmed cell death-1; PD-L1, programmed death-ligand 1; TPS, tumor
- 4 positivity score.