

OMTO, Volume 17

Supplemental Information

B7-H3-Targeted CAR-T Cells

Exhibit Potent Antitumor Effects

on Hematologic and Solid Tumors

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Table S1. B7-H3 expression patterns in human tumour tissues analysed with IHC

Tumor Type	#Stained	Positive (%)	Intensity			
			- (%)	+ (%)	++ (%)	+++ (%)
Bladder: BLCA	16	88	12	19	25	44
Breast: BRCA	10	60	40	10	20	30
Cervical: CESC	14	57	43	36	14	7
Colorectal: COAD	16	44	56	12	19	13
Esophageal: ESCA	9	89	11	11	11	67
Stomach: STAD	8	63	37	13	25	25
Kidney: KIRP	10	60	40	10	40	10
Liver: LIHC	15	80	20	13	40	27
Lung: LUSC	21	76	24	18	29	29
Lymphoma: DLBC	7	71	29	42	29	0
Skin: SSCC	20	80	20	15	15	50
Ovarian: OV	11	64	36	27	18	19
Pancreatic: PAAD	23	61	39	17	13	31
Prostate: PRAD	18	50	50	11	6	33
Testis: ANSE and SEMI	5	20	80	20	0	0
Testis: EMCA	4	100	0	50	50	0
Tongue: ERMS	2	0	100	0	0	0
Total tumor tissue:	209	66	34	18	21	27

Note: The expression levels were scored as no expression (-), < 20% positive cells (+, low or weak expression), 20-50% positive cells (++, moderate expression), and > 50% positive cells (+++, high or strong expression).

BLCA: Bladder Urothelial Carcinoma, BRCA: Breast invasive carcinoma, CESC: Cervical squamous cell carcinoma, COAD: Colon adenocarcinoma, ESCA: Esophageal carcinoma, STAD: Stomach adenocarcinoma, KIRC: Kidney renal clear cell carcinoma, LIHC: Liver hepatocellular carcinoma, LUSC: Lung squamous cell carcinoma, DLBC: Diffuse Large B-cell Lymphoma, SSCC: Skin squamous cell carcinoma, OV: Ovarian serous cystadenocarcinoma, PAAD: Pancreatic adenocarcinoma, PRAD: Prostate adenocarcinoma, ANSE: Anaplasia seminoma, SEMI: seminoma, EMCA: Embryonal carcinoma, ERMS: Embryonal rhabdomyosarcoma.

Table S2. B7-H3 expression patterns in human TATs analysed with IHC

Tumor Type	#Stained	Positive (%)	Intensity			
			- (%)	+ (%)	++ (%)	+++ (%)
Bladder (TAT)	16	19	81	19	0	0
Breast (TAT)	10	10	90	10	0	0
Cervical (TAT)	14	29	71	29	0	0
Colorectal (TAT)	16	63	38	44	19	0
Esophageal (TAT)	9	33	67	0	33	0
Stomach (TAT)	8	63	38	13	25	0
Kidney (TAT)	10	0	100	0	0	0
Liver (TAT)	15	53	47	27	27	0
Lung (TAT)	21	52	48	29	24	0
Lymphoma (TAT)	7	29	71	14	0	0
Skin (TAT)	20	55	45	25	30	0
Ovarian (TAT)	11	45	55	27	18	0
Pancreatic (TAT)	23	35	65	17	17	0
Prostate (TAT)	18	33	67	22	11	0
Testis (TAT)	9	56	44	56	0	0
Placenta (TAT)	2	100	0	0	100	0
Total TAT:	209	84	125	23	16	0

Note: TAT: tumor adjacent tissues; The expression levels were scored as no expression (-), < 20% positive cells (+, low or weak expression), 20-50% positive cells (++, moderate expression), and > 50% positive cells (+++, high or strong expression).

Table S3. B7-H3 expression patterns in human normal tissues analysed with IHC

Tumor Type	#Stained	Positive (%)	Intensity			
			- (%)	+ (%)	++ (%)	+++ (%)
Breast	5	20	80	20	0	0
Cerebellum	8	0	100	0	0	0
Cerebrum	8	13	87	13	0	0
Esophagus	6	17	83	17	0	0
Stomach	6	0	100	0	0	0
Intestine	8	0	100	0	0	0
Colon	4	25	75	25	0	0
Heart	4	0	100	0	0	0
Kidney	8	0	100	0	0	0
Liver	12	67	33	42	25	0
Lung	9	0	100	0	0	0
Ovary	5	60	40	60	0	0
Pancreas	6	17	83	17	0	0
Prostate	8	38	62	25	13	
Skin	10	0	100	0	0	0
Testis	4	50	50	50	0	0
Thyroid	9	0	100	0	0	0
Tonsils	5	20	80	20	0	0
Uterus	7	43	57	14	29	0
Adrenal gland	15	40	60	20	20	0
Spleen	6	0	100	0	0	0
Bladder	8	13	87	13	0	0
Larynx	7	14	86	14	0	0
Salivary gland	5	20	80	20	0	0
Total tumor tissue:	173	20	80	14	6	0

Note: The expression levels were scored as no expression (-), < 20% positive cells (+, low or weak expression), 20-50% positive cells (++, moderate expression), and > 50% positive cells (+++, high or strong expression).

Table S4. Affinity analysis of mAb-J42 and J42-scFv-Fc with B7-H3-ECD-His

Antibody	Ka (1/MS)	Kd (1/s)	KD(pM)
mAb-J42	5.619×10^5	3.729×10^{-5}	66.36
J42-scFv-Fc	1.957×10^5	2.515×10^{-5}	128.5

Note: The binding affinity was measured using the BiacoreTM X100 instrument with CM5 sensor chip (GE Healthcare). mAb-J42 and Fc-tagged J42-scFv were indirectly captured onto the surface, and the B7-H3-ECD-His protein was injected across the chip in a twofold dilution series. The equilibrium dissociation constant (KD) was obtained using BIAevaluation 2.0 software.

Table S5. B7-H3 expression in tumor cell lines

Cell line	Tumor type	B7-H3	Cell line	Tumor type	B7-H3
A375	melanoma	+	SKOV3	ovarian cancer	+
DU145	prostate cancer	+	H1975	lung cancer	+
SW480	colon cancer	+	MDA-MB-231	breast cancer	+
786-O	renal cancer	+	HepG2	liver cancer	+
PANC-1	pancreatic cancer	+	SiHa	cervical cancer	+
U266	multiple myeloma	+	THP-1	monocytic leukemia	+
MV4-11	B myelomonocytic leukemia	+	U937	histiocytic lymphoma	+
K562	myelogenous leukemia	+	HL-60	promyelocytic leukemia	+
Jurkat	T cell leukemia	-	Raji	Burkitt's lymphoma	-
Daudi	Burkitt's lymphoma	-	Ramos	Burkitt's lymphoma	-

Note: +, positive; -, negative.

Table S6. Characteristics of AML

AML Sample ID	Age	Sex	Cytogenetics	B7-H3% positive
1	10 years	male	t(3;11), MLL-R	80.4
2	8 years	male	t(6;11), MLL-R	73.0
3	15 years	female	PML/RARA+	77.2
4	22 years	male	inv(3)(q21.3q26.2)	45.7
5	15 years	male	46, XY	40.5
6	6 years	male	t(4;11), MLL-R	42.1
7	7 years	female	PML/RARA+	38.8
8	31 years	male	46, XY	60.2

Note: Bone marrow aspirates from patients with monocytic/myelomonocytic AML were obtained and subjected to flow cytometry assay.

Fold Change Between Tumor and Normal

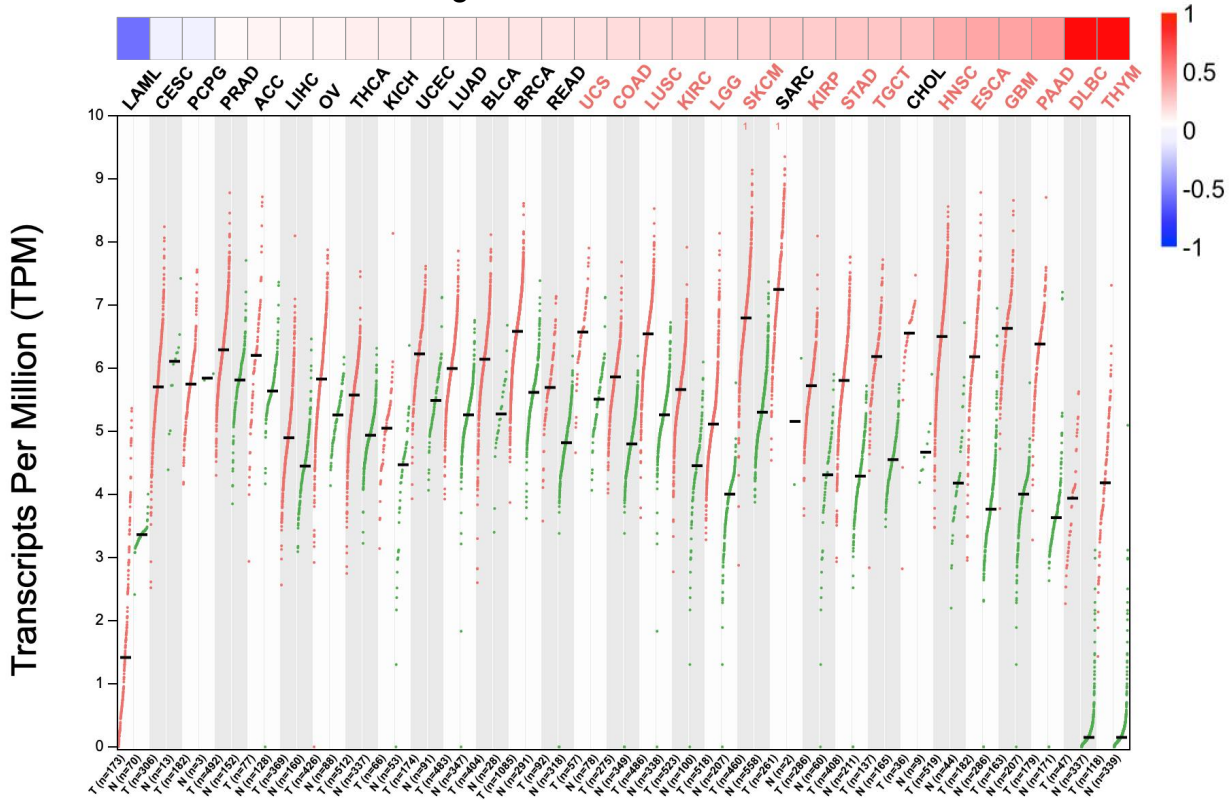


Figure S1. B7-H3 expression analysis using GEPIA

Expression level of B7-H3 in 9433 tumor samples and 5540 normal samples from the TCGA and the GTEx projects including 31 tumor subtypes were analyzed using GEPIA web server (<http://gepia.cancer-pku.cn>).

The upper color indicator bar indicates the expression fold changes in tumor samples compared to normal samples.

Tumor subtypes shown in red font represent statistically significant upregulation in tumor samples ($p < 0.05$).

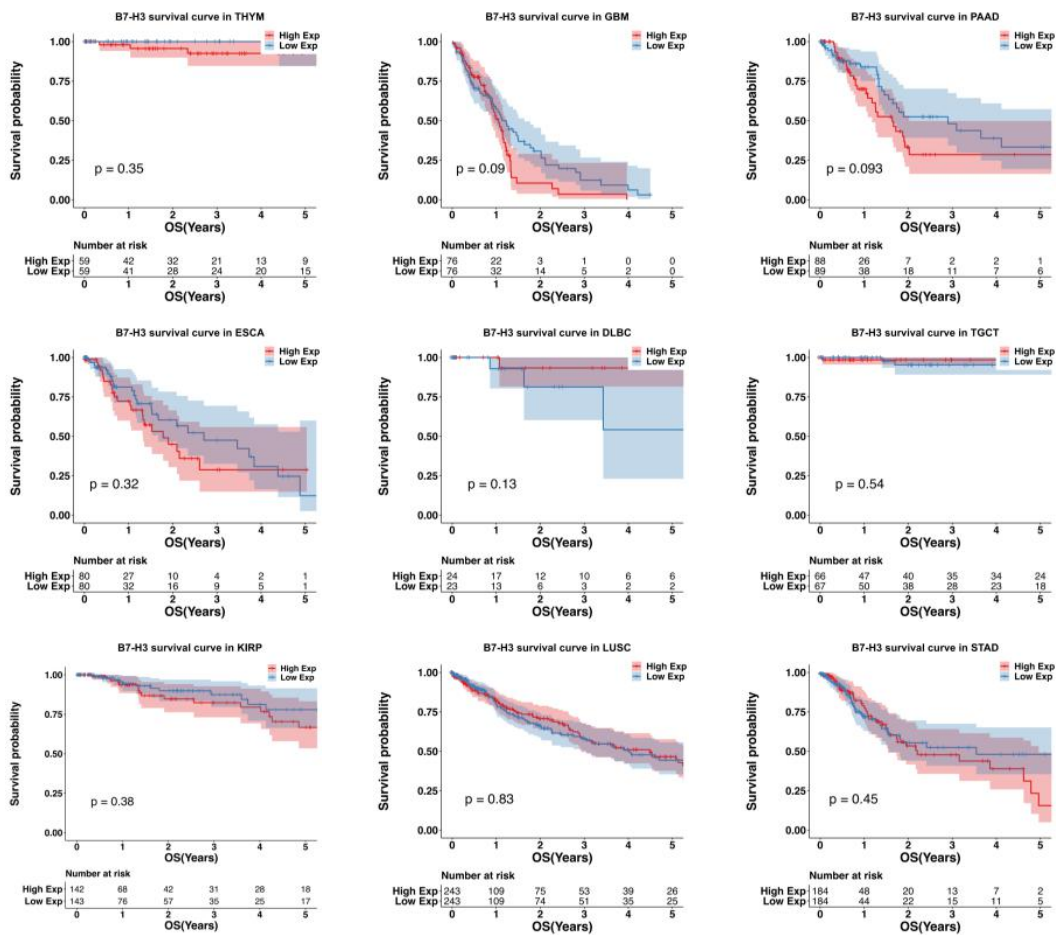


Figure S2. Correlation analysis between B7-H3 expression and survival using GEPIA

Correlation analysis between B7-H3 expression and survival across 9 common tumor subtypes (THYM, GBM, PAAD, ESCA, DLBC, TGCT, KIRP, LUSC and STAD) were performed by using GEPIA web server (<http://gepia.cancer-pku.cn>).

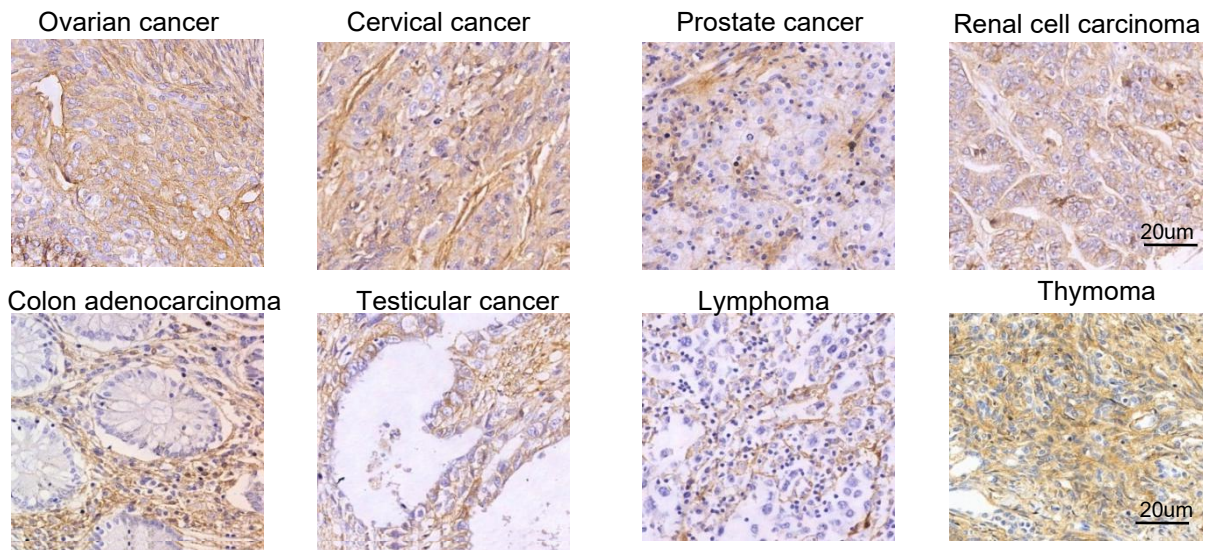


Figure S3. IHC staining for B7-H3 expression in human tumor tissues

Representative IHC images of B7-H3 expression in human cancer tissues including ovarian cancer, cervical cancer, prostate cancer, renal cell carcinoma, colon adenocarcinoma, testicular cancer, lymphoma and thymoma.

Scale bars, 20 μ m.

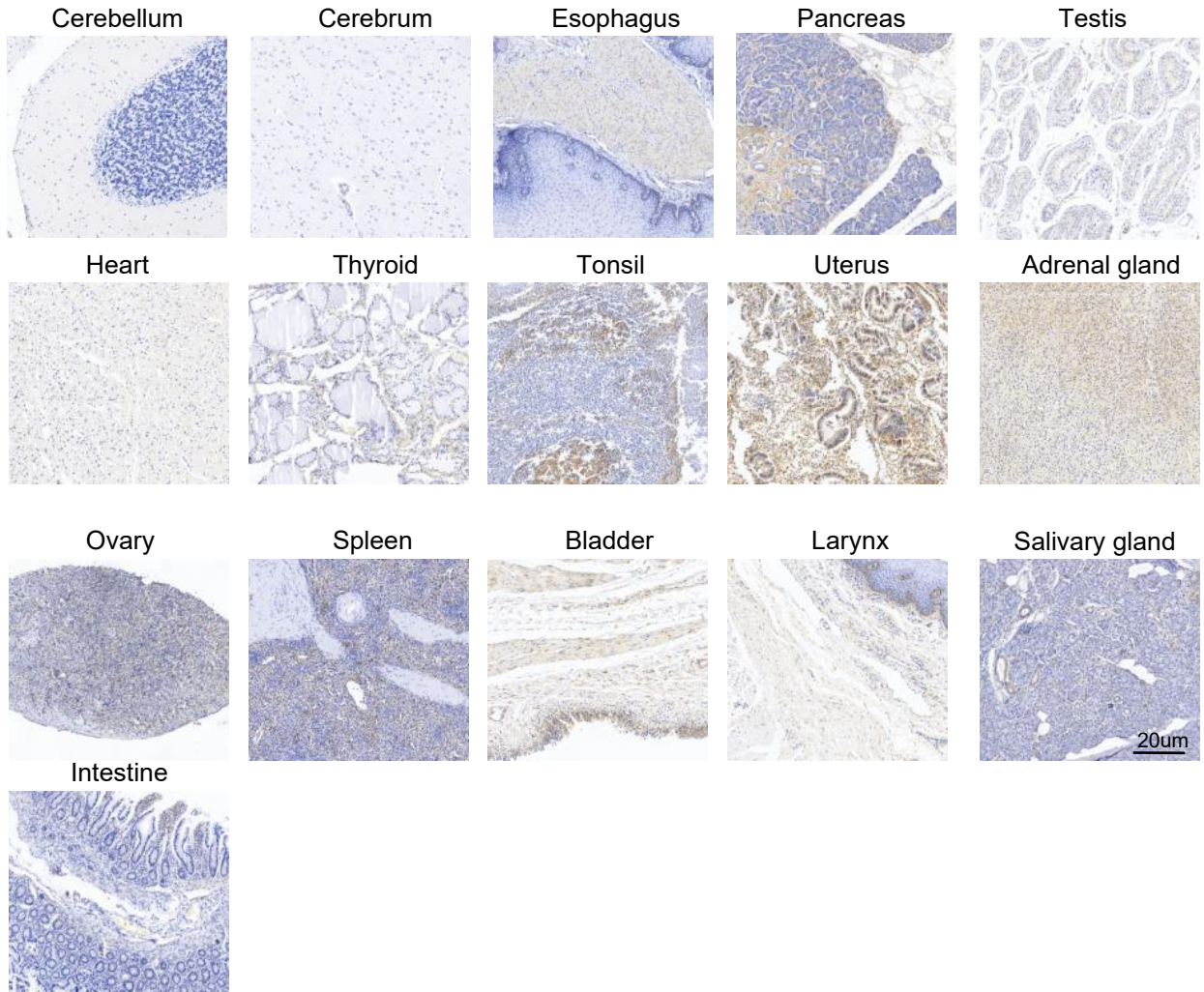


Figure S4. IHC staining for B7-H3 expression in human normal tissues

Representative IHC images of B7-H3 expression in human normal tissues including cerebellum, cerebrum, esophagus, pancreas, testis, heart, thyroid, tonsil, uterus, adrenal gland, ovary, spleen, bladder, larynx, salivary gland and intestine. Scale bars, 200 µm.

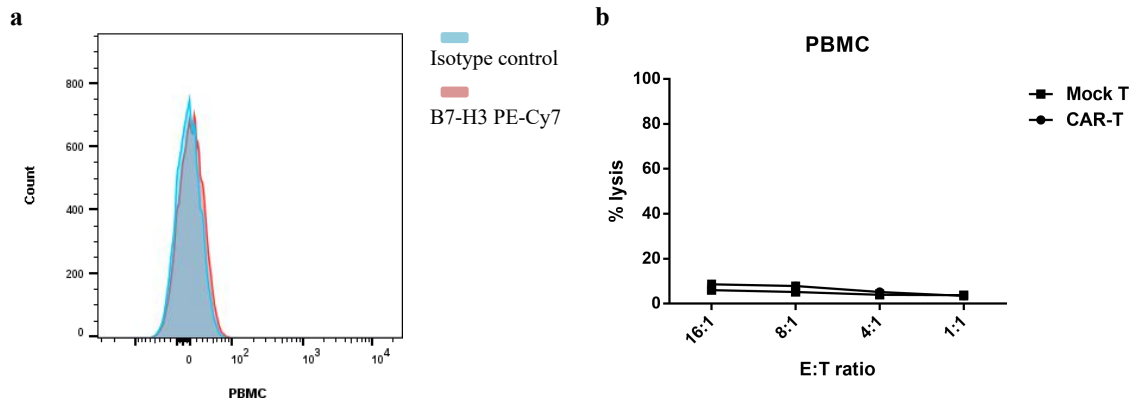


Figure S5. Expression of B7-H3 in PBMCs and the cytotoxicity of CAR-T cells against PBMCs

a. Expression of B7-H3 in PBMCs was evaluated by FACS. Cells were incubated with B7-H3-PE-Cy7 (purchased from BioLegend) (red) or its corresponding isotype control (blue).

b. ^{51}Cr -release assay to measure the cytotoxicity of CAR-T cells against PBMCs at different E:T ratios.