OMTO, Volume *18*

Supplemental Information

Chimeric Antigen Receptor T Cell Therapy Targeting ICAM-1 in Gastric Cancer

Minkyu Jung, Yanping Yang, Jaclyn E. McCloskey, Marjan Zaman, Yogindra Vedvyas, Xianglan Zhang, Dessislava Stefanova, Katherine D. Gray, Irene M. Min, Raza Zarnegar, Yoon Young Choi, Jae-Ho Cheong, Sung Hoon Noh, Sun Young Rha, Hyun Cheol Chung, and Moonsoo M. Jin

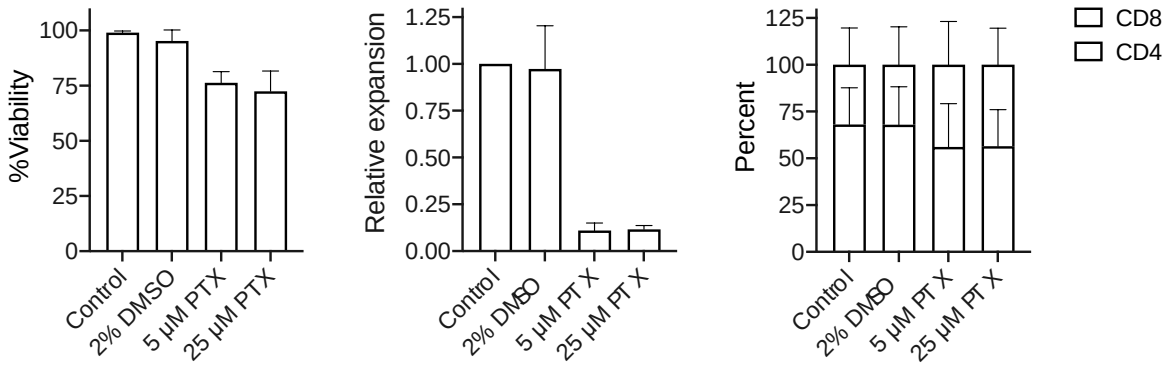

Figure S1. The effect of paclitaxel on CAR T cell viability and expansion
ICAM-1 CAR T cells were incubated with or without paclitaxel (5 or 25 μM in 2% DMSO) in culture media. After 4 hours incubation, T cells were washed and resuspended in fresh culture media. At 72 hours after incubation with paclitaxel, the viability, cell number, and CD4/CD8 ratios of T cells were analyzed. Data represent mean ± SD of three batches of CAR T cells (CP1, CP5, and CP8).

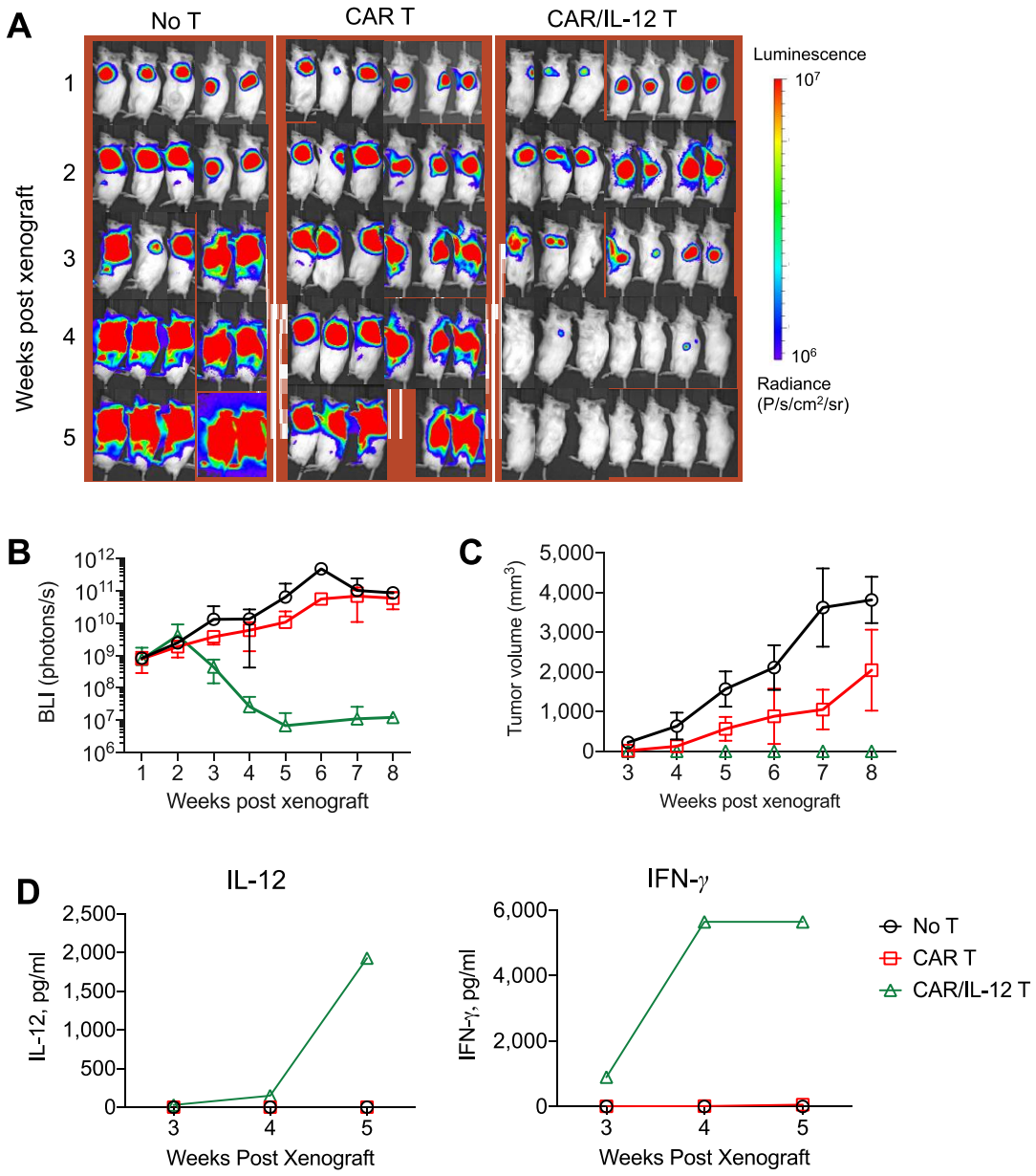

Figure S2. Inducible IL-12 secretion enhances anti-tumor activity of CAR T cells against pre-established solid tumor

NSG mice were subcutaneously implanted with 1×10^6 GFP^+FLuc^+ 8505C cells in the upper left flank. Five days after tumor inoculation, mice were either left untreated (no T) or treated with CAR T or CAR/IL-12 T cells (10×10^6 cells/mouse) via tail vein injection. (**A**) Whole-body bioluminescence imaging was used to evaluate tumor growth. (**B**) Quantitation of whole-body bioluminescence intensity. Results were pooled from two independent experiments using two different batches of CAR T cells, and donor-matched CAR/IL-12 T cells. Data represent mean ± SD (n = 5–7 mice per cohort). (**C**) Tumor volume measurements over time. Data are shown as mean ± SD (n = 5–7). (**D**) Serum IL-12 and IFN-γ levels were measured from blood collected at 3, 4, and 5 weeks after tumor xenograft. IFN-γ readouts of CAR/IL-12 T samples at 4 and 5 week timepoints were above the upper limit of detection.

Table S1. Baseline characteristics of patients according to ICAM-1 expression

ICAM-1 intensity	0	1 (light brown)	2 (brown)	3 (dark brown)
stage II (n = 54)	42 (77.8%)	4 (7.4%)	5 (9.3%)	3 (5.6%)
stage III (n = 80)	41 (51.2%)	17 (21.3%)	16 (20%)	6 (7.5%)

Table S2. Summary of xenograft engraftments and time to death by intravenous and intraperitoneal injection of GC cell lines

Cell line	TCGA	Lauren	ICAM-1	i.v. injection[a]		i.p. injection[b]	
				Engraftment pattern	Time to death (days)	Engraftment pattern	Time to death (days)
MKN-28	CIN	Intestinal	High	Intrathoracic & Intraperitoneal	90	Intraperitoneal	60
SNU-5	CIN	Diffuse	Negative	Intraperitoneal	80	Intraperitoneal	70
SNU-719	EBV	Intestinal	Moderate	Intrathoracic & Intraperitoneal	90	Intraperitoneal	100
NCC-24	EBV	Diffuse	Moderate	Intraperitoneal	100	Intraperitoneal	100
SNU-638	MSI	Intestinal	High	Intraperitoneal	70	Intraperitoneal	50
SNU-1	MSI	Diffuse	Negative	Intraperitoneal	100	Intraperitoneal	100
HS746t	GS	Diffuse	High	Intrathoracic & Intraperitoneal	50	Intraperitoneal	70
SNU-601	GS	Diffuse	Moderate (Broad)	Intrathoracic & Intraperitoneal	60	Intraperitoneal	100

[a]7.5×10^5 tumor cells injected intravenously; [b]3×10^6 tumor cells injected intraperitoneally.

Table S3. Summary of pre-infusion ICAM-1 CAR T cell characteristics

CAR T	Donor no.	Methods	CD4+ (%)	CD8+ (%)	c-Myc+/SSTR2+ (%)
CP1	1	Prodigy	87	11	52
CP5	5	Prodigy	65	34	45
CP9	9	Prodigy	42	57	57
CAR/IL-12	9	Rolling tube	42	53	8

Table S4. Summary of post-infusion ICAM-1 CAR T cell characteristics

CAR T	Tumor model[a]	Blood collection[b]	CD3+	CD4: CD8	c-Myc+	T_{EM} ($CCR7^-$ /$CD45RA^-$)[c]
CP9	SNU-638, i.p.	8	19.0%	1:2.6	ND	ND
CAR/IL-12	SNU-638, i.p.	6	79.4%	1:2.0	13.3%	89.7%
CAR/IL-12	8505C, s.c.	5	83.2%	1:1.2	11.2%	93.3%

[a]SNU-638, gastric cancer; 8505C, thyroid cancer; IP, intraperitoneal tumor model; s.c., subcutaneous tumor model. [b]Weeks post xenograft. [c]Effector memory T cell population (T_{EM}) were determined by anti-CCR7 and CD45RA antibody binding. ND, not determined.