

Supplementary Material 1.

Supplementary results of A Clinical Study of Autologous Chimeric Antigen Receptor Macrophage Targeting Mesothelin in Ovarian Cancer Therapy.

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Fig. S1. Structure of the CAR used in the SY001 infusion product.

Fig. S2. Characteristics and in vitro anti-ovarian cancer function of SY001 CAR-pMACs.

Fig. S3. Mesothelin expression on HO8910 and SKOV3 cells.

Table S1. Characteristics of SY001 used for the two patients.

Table S2. Patient characteristics and disease progression.

Fig. S1. Structure of the CAR used in the SY001 infusion product.

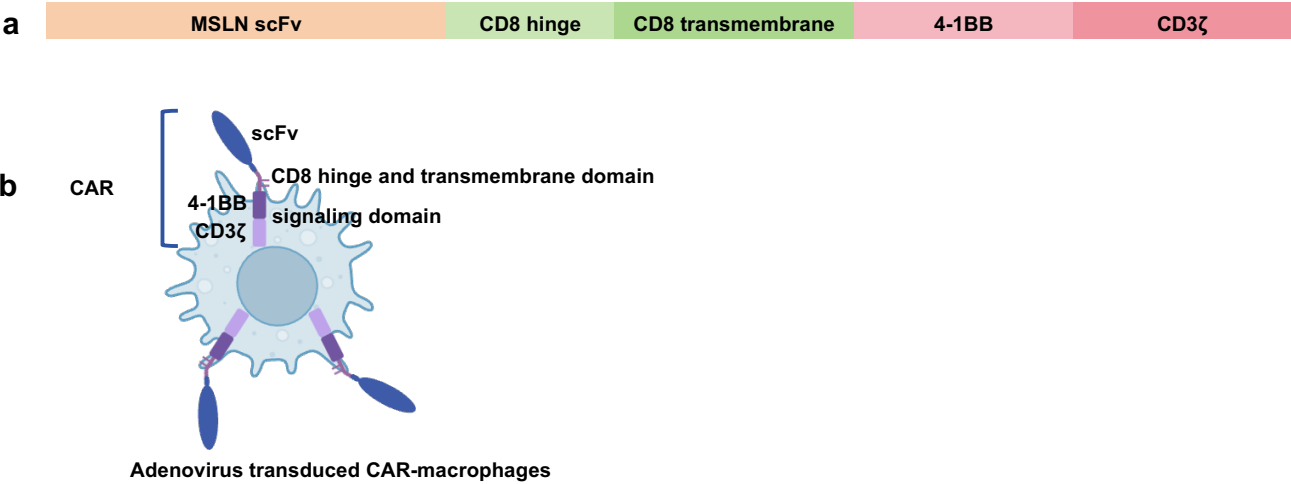


Fig S1. Structure of the CAR used in the SY001 infusion product.

a and b. The structure of SY001 CAR consists of a single-chain antibody fragment (scFv) that targets mesothelin, a CD8 hinge and transmembrane domain, a 41BB co-stimulatory domain, and a CD3 ζ signaling domain.

Fig. S2. Characteristics and in vitro anti-ovarian cancer function of SY001 CAR-pMACs.

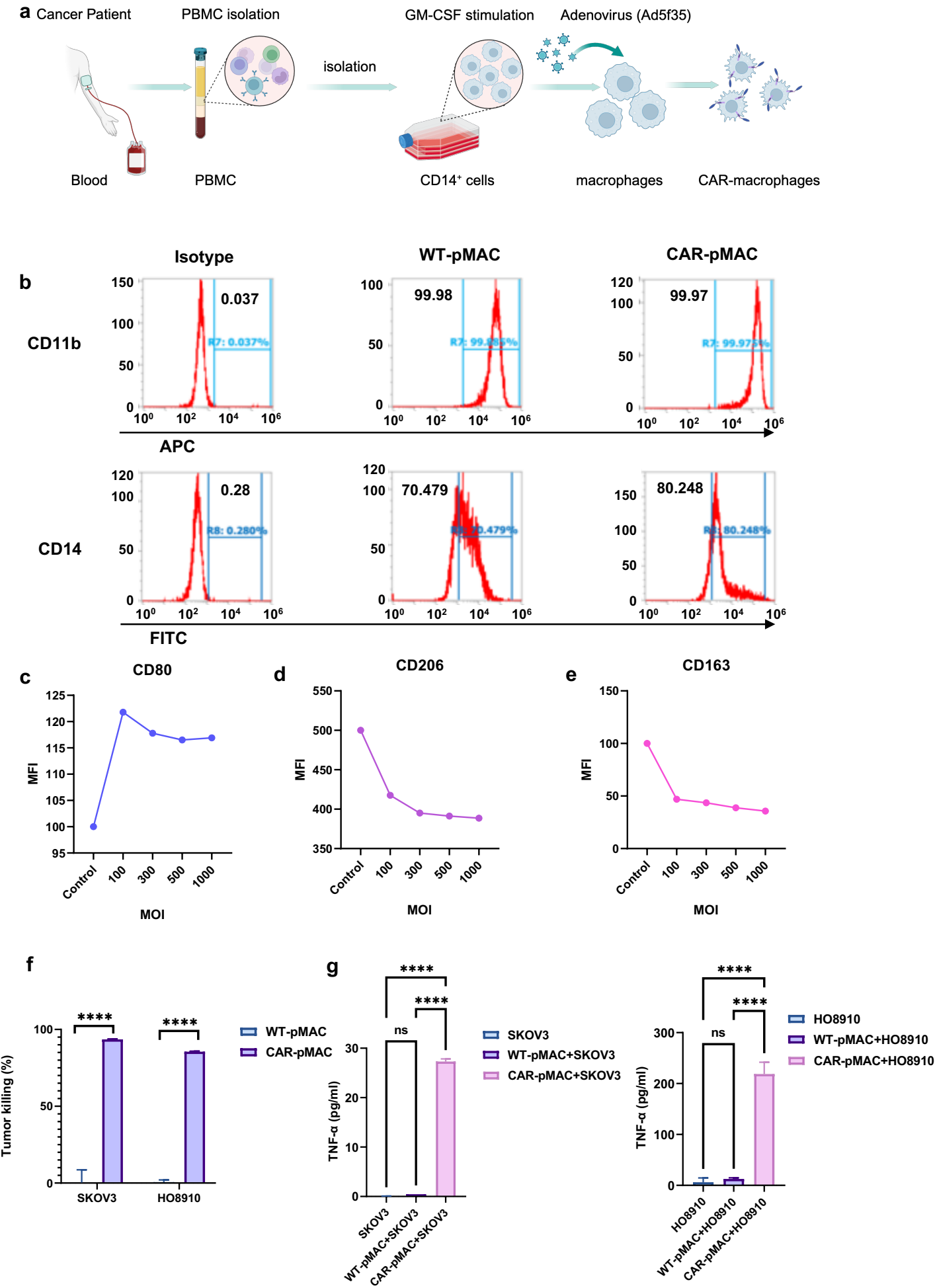


Fig. S2. Characteristics and in vitro anti-ovarian cancer function of SY001 CAR-pMACs.

a. The flow diagram of SY001 CAR-pMACs production from cancer patients.

b. The expression of CD11b (up) and CD14 (down) on WT-pMACs (middle) and Ad5f35 adenovirus transduced CAR-pMACs (right) was detected by flow cytometry. This experiment has been repeated for three times with similar results.

c-e. The expression of CD80 (c), CD206 (d) and CD163 (e) on pMACs transduced with Ad5f35 adenovirus at different multiplicity of infection (MOI) was detected by flow cytometry.

f. Luciferase assays showing pMAC cytotoxicity against cancer cells when co-cultured with SKOV3 or HO8910 cells for 72 h at an effector-to-target ratio (E:T) of 20:1. (n=3 biologically independent samples). Statistics by unpaired t test. The luciferase gene was introduced into the tumor cells via lentivirus and expressed within the tumor cells, allowing for the measurement of tumor cell viability using D-luciferin sodium salt in the luciferase assay.

g. The levels of TNF- α in the medium of pMAC-SKOV3 (left) and pMAC-HO8910 (right) co-cultures were measured after 72 hours of co-culturing (E:T=20:1). Data represent three biologically independent samples (n=3). Statistical analysis was conducted using a one-way ANOVA test.

f and g, Data was shown as mean $\pm$ SD. ****P < 0.0001; ns, not significant.

Fig. S3. Mesothelin expression on HO8910 and SKOV3 cells.

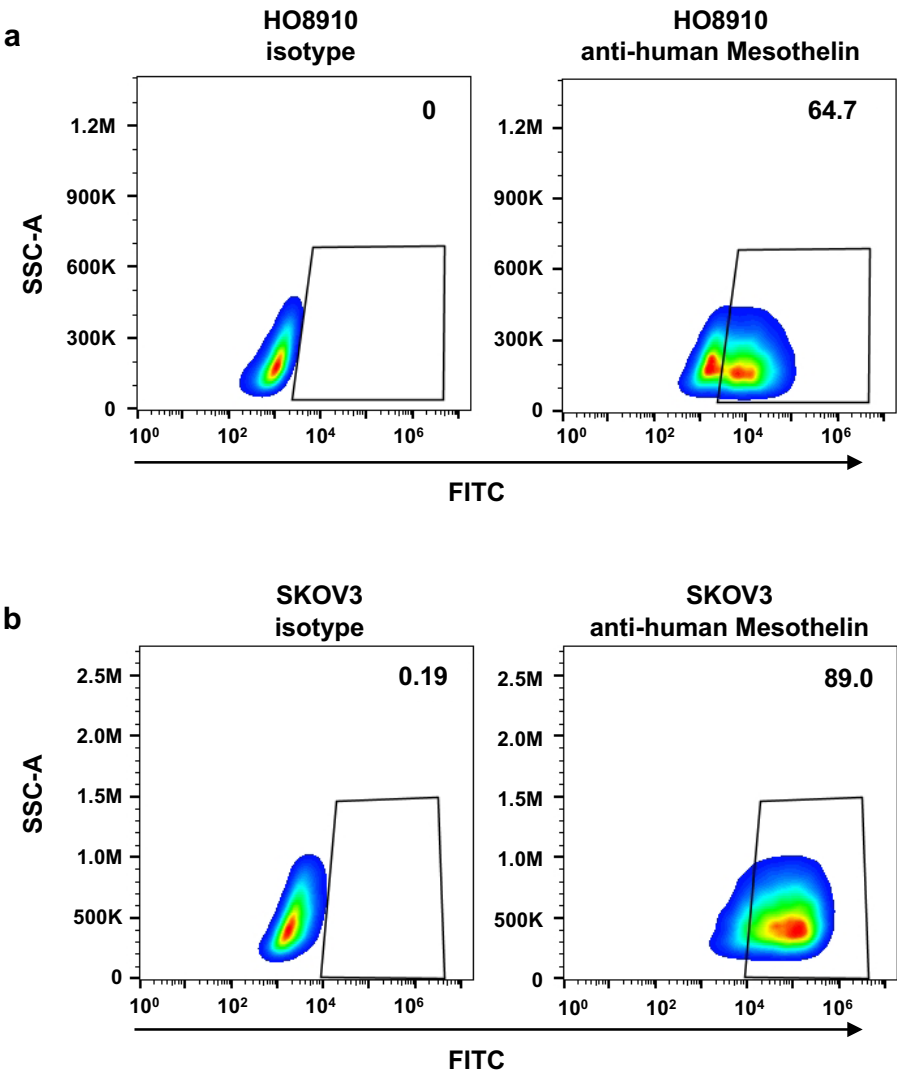


Fig. S3. Mesothelin expression on HO8910 and SKOV3 cells.

- a.** Flow cytometry analysis of HO8910 cells stained with an isotype FITC antibody (left) or an anti-human mesothelin FITC antibody (right).
- b.** Flow cytometry analysis of SKOV3 cells stained with an isotype FITC antibody (left) or an anti-human mesothelin FITC antibody (right).

Table S1. Characteristics of SY001 used for the two patients.

	Patient 1 (S01001/001)	Patient 2 (S01002/002)
Cell viability	82.23%	73.12%
MSLN CAR expression	79.12%	80.35%
CD11b	93.65%	80.11%
CD14	94.47%	74.07%
CD3	1.54%	8.87%
CD19	1.05%	4.51%
Sterility	negative	negative
Endotoxin	less than 5.0EU/ml	less than 5.0EU/ml
Mycoplasma	negative	negative

Table S2. Patient characteristics and disease progression

Subjects		Target lesion site		Total diameter (mm)	Non-target lesion site	New lesions	Target lesion evaluation	Non-target lesion evaluation
Single-dose group - 001	Baseline period	1.	Lymph nodes: left axillary lymph nodes are enlarged, the short diameter of the lymph nodes is 82mm.	113	Lymph nodes: multiple enlarged lymph nodes are present in the neck, supraclavicular region, left axilla, retroperitoneum of the abdominal cavity, and pelvic cavity	NA	NA	NA
		2.	Lymph nodes: retroperitoneal lymph nodes are enlarged, and the short diameter of the lymph nodes is 31mm.					
	D28	1.	Lymph nodes: left axillary lymph nodes are enlarged, the short diameter of the lymph nodes is 93mm.	124	Lymph nodes: multiple enlarged lymph nodes are present in the neck, supraclavicular region, left axilla, retroperitoneum of the abdominal cavity, and pelvic cavity	New spots and nodules appeared in both lungs compared to before	SD	Non-CR/Non-PD
		2.	Lymph nodes: retroperitoneal lymph nodes are enlarged, and the short diameter of the lymph nodes is 31mm.					
Multiple-dose group-001	Baseline period	1.	Lymph nodes: left axillary lymph nodes are enlarged, and the short diameter of the lymph nodes is 93mm.	124	Lymph nodes: multiple enlarged lymph nodes are present in the neck, supraclavicular region, left axilla, retroperitoneum of the abdominal cavity, and pelvic cavity	NA	NA	NA
		2.	Lymph nodes: retroperitoneal lymph nodes are enlarged, and the short diameter of the lymph nodes is 31mm.					
	D28	1.	Lymph nodes: left axillary lymph nodes are enlarged, and the short diameter of the lymph nodes is 95mm.	126	Lymph nodes: multiple enlarged lymph nodes are present in the neck, supraclavicular region, left axilla, retroperitoneum of the abdominal cavity, and pelvic cavity	Multiple nodular lesions and enhancing lesions newly appeared in the left dorsal sphincter and anterior chest wall	iSD	Non-iCR
		2.	Lymph nodes: retroperitoneal lymph nodes are enlarged, and the short diameter of the lymph nodes is 31mm.					
Multiple-dose group-002	Baseline period	1.	Lymph nodes: right supraclavicular lymph nodes are enlarged, and the short diameter of the lymph nodes is 18mm;	33	1. In the pelvic cavity: strip-like shadows exist in the pelvic cavity 2. Lymph nodes: multiple lymph nodes of different sizes exist in the retroperitoneum of the abdominal cavity and the pelvic cavity 3. Lymph nodes: multiple lymph nodes of different sizes exist in the bilateral supraclavicular region; the peritoneum, omentum and mesentery are thickened, and flocculent shadows exist 4. Lymph nodes: Lymph nodes exist in the pericardial diaphragm region	NA	NA	NA
		2.	Lymph nodes: retroperitoneal lymph nodes are enlarged, and the short diameter of the lymph nodes is 15mm					
	D28	1.	Lymph nodes: right supraclavicular lymph nodes are enlarged, and the short diameter of the lymph nodes is 21mm;	36	1. In the pelvic cavity: strip-like shadows exist in the pelvic cavity 2. Lymph nodes: multiple lymph nodes of different sizes exist in the retroperitoneum of the abdominal cavity and the pelvic cavity 3. Lymph nodes: multiple lymph nodes of different sizes exist in the bilateral supraclavicular region; the peritoneum, omentum and mesentery are thickened, and flocculent shadows exist 4. Lymph nodes: Lymph nodes exist in the pericardial diaphragm region	1. Multiple enhanced nodular shadows are newly appeared in the liver, and the largest of which is about 17×18mm;	SD	Non-CR/Non-PD
		2.	Lymph nodes: retroperitoneal lymph nodes are enlarged, and the short diameter of the lymph nodes is 15mm			2. Newly appeared pelvic lymphadenopathy		