Electronic Supplementary Information (ESI)

A potential platform of combining sialic acid derivative-modified paclitaxel cationic liposomes with antibody-drug conjugates inspires robust tumor-specific immunological memory in solid tumors

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Tab.S1 Characterization comparison of PTX cationic liposomes PTX-CL and PTX-SAL (with DEPC phospholipids) prepared in this research with the PTX cationic liposomes referring to the Phase III clinical product EndoTAG-1® prescription (with DOPC phospholipids). After optimizing the prescription, the use of DEPC phospholipids can increase the Drug-Loading Efficiency of PTX. The data are represented as mean ± SD, $n = 3$.

	PTX-CL	PTX-SAL	PTX-CL-DOPC	PTX-SAL-DOPC
Size (nm)	113.6 ± 0.6	106.5 ± 0.1	119.0 ± 0.2	117.0 ± 0.1
PDI	0.173 ± 0.008	0.059 ± 0.007	0.054 ± 0.008	0.088 ± 0.003
Zeta potential (mV)	30.8 ± 1.5	22.6 ± 0.7	27.7 ± 0.9	24.2 ± 0.9
Encapsulation Efficiency (%)	95.4 ± 2.3	96.9 ± 2.5	93.0 ± 1.1	95.7 ± 1.7
Drug-Loading Efficiency (%)	3.6 ± 0.1	3.5 ± 0.1	2.8 ± 0.2	2.7 ± 0.3

Tab.S2 The concentration calculation of the payload MMAE in the antitumor experiment. In the antitumor experiment, the dosage of ADC 10 mg/kg referred to the concentration of whole ADC. Under this ADC dosage, the MMAE concentration of Mabwell's ADC product (ref-MMAE-20200528) and the original product EV (PADCEV_102374) was calculated and compared. Molecular weights of ref-MMAE-20200528, PADCEV_102374, and MMAE were 153316 g/mol, 153316 g/mol, and 717.98 g/mol, respectively.

	ref-MMAE-20200528	PADCEV_102374
Molecular weight (g/mol)	153316	153316
Administration dosage of whole ADC (mg/kg)	10	10
Administration dosage of whole ADC (mol/kg)	6.5×10^ (-8)	6.5×10^ (-8)
MMAE concentration (mol/kg)	2.6×10^ (-7)	2.6×10^ (-7)
MMAE concentration (mg/kg)	0.19	0.19

Tab.S3 The Drug-to-Antibody Ratio (DAR) of two ADC products was determined by hydrophobic interaction chromatography-high performance liquid chromatography (HIC-HPLC) spectrophotometry. The average DAR of ref-MMAE-20200528 was 4.003, while the average DAR of PADCEV_102374 was 4.024 according to the HIC-HPLC analysis.

The number of coupling drugs	Percentage area of chromatographic peak	
	ref-MMAE-20200528	PADCEV_102374
DAR=0	0.55	1.53
DAR=1	0	0.85
DAR=2	28.58	26.47
DAR=3	0.1	1.33
DAR=4	46.05	42.82
DAR=5	0.54	1.69
DAR=6	17.73	18.37
DAR=7	0	1.33
DAR=8	6.46	5.34

Note:

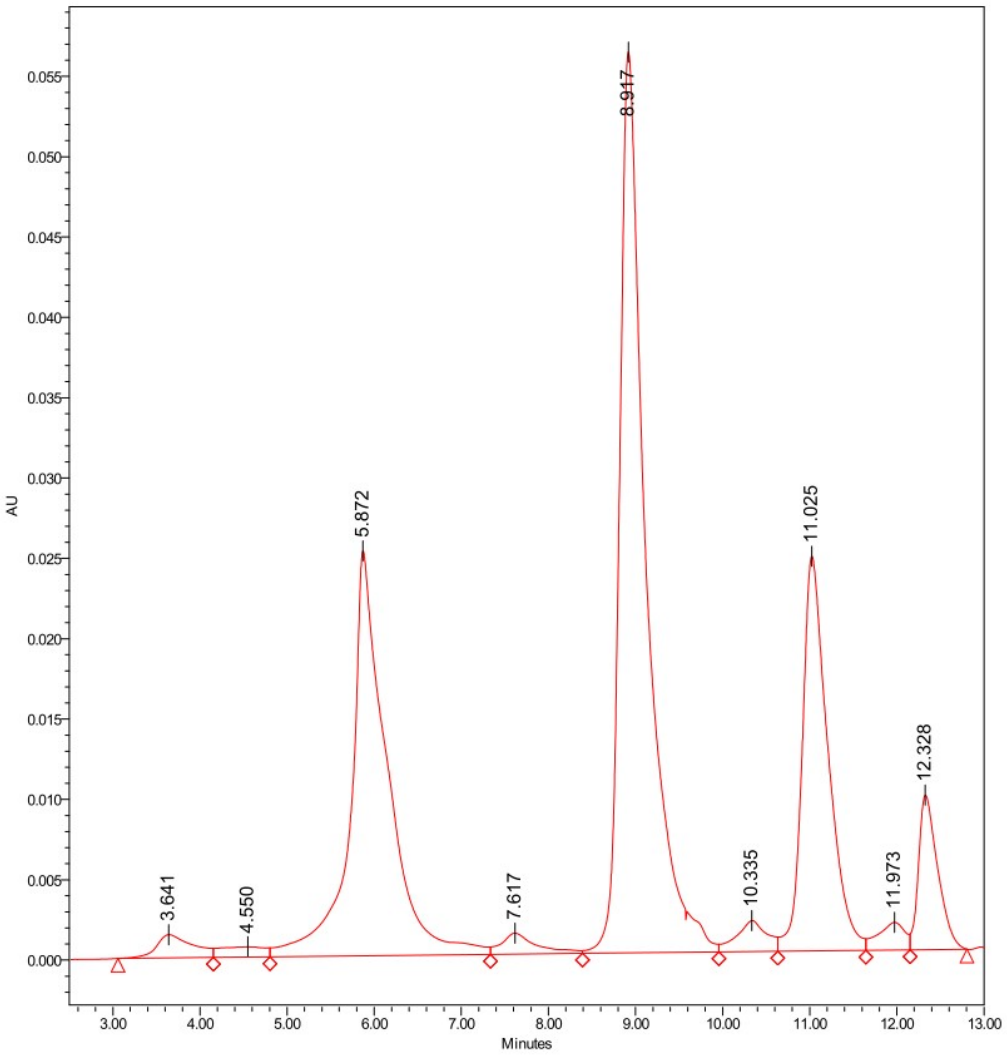
(a) The difference in data was the fluctuation of measurement and there was no statistically significant.

(b) The data in this Tab.S3 was organized according to the chromatograms of Fig.S1 and Fig.S2 including the chromatographic peak area of each drug loading. The HIC-HPLC spectrum of both two ADC products displayed 5 major peaks, corresponding to 0, 2, 4, 6, and 8 drugs per antibody (DAR=0, DAR=2, DAR=4, DAR=6, DAR=8).

(c) The weighted average DAR value was calculated by the percentage area of the chromatographic peak and the number of coupling drugs. DAR = Σ(Weighted peak area)/100 = Σ(relative peak area × number of coupling drugs)/100. The average DAR of ref-MMAE-20200528 was 4.003, while the average DAR of PADCEV_102374 was 4.024 according to the HIC-HPLC analysis.

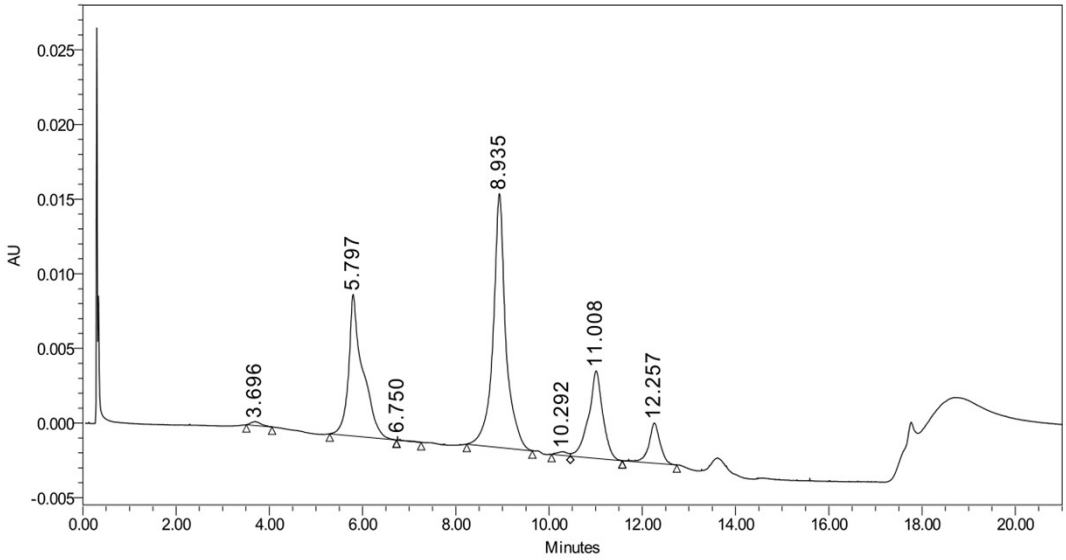
(d) HIC-HPLC experiments were performed using Sepax Proteomix HIC Butyl-NP5 (5 μm, 4.6 mm×35 mm). The mobile phase was a gradient of (A) 25 mM phosphate with 1.2 M ammonium sulfate (pH 7.0); (B) 25 mM phosphate (pH 7.0); and (C) 100% isopropyl alcohol at a flow rate of 0.8 mL/min. The column temperature was at 30 ^{o}C, and 20 μL of injection volume was detected at a wavelength of 280 nm.

Fig.S1 Conjugated drug distribution by HIC-HPLC of original products EV (PADCEV_102374).



	SampleName	Retention Time (min)	Area	% Area	Height
1	PADCEV_102374	3.641	42187	1.53	1445
2	PADCEV_102374	4.550	23329	0.85	643
3	PADCEV_102374	5.872	736767	26.74	25195
4	PADCEV_102374	7.617	36621	1.33	1306
5	PADCEV_102374	8.917	1179598	42.82	56059
6	PADCEV_102374	10.335	46455	1.69	1926
7	PADCEV_102374	11.025	505969	18.37	24557
8	PADCEV_102374	11.973	36745	1.33	1721
9	PADCEV_102374	12.328	147219	5.34	9619

Fig.S2 Conjugated drug distribution by HIC-HPLC of ADC provided by Mabwell (Shanghai) Bioscience Co., Ltd. (ref-MMAE-20200528).



	RT	Area	% Area	Height	USP Plate Count	USP Resolution (HH)
1	3.696	3839	0.55	260	1.523039e+003	
2	5.797	200385	28.58	9502	1.761436e+003	4.848385e+000
3	6.750	696	0.10	248	2.186532e+006	3.923925e+000
4	8.935	322943	46.05	17008	6.866355e+003	1.023559e+001
5	10.292	3771	0.54	241		
6	11.008	124328	17.73	5865	6.526553e+003	
7	12.257	45274	6.46	2702	1.138033e+004	2.804342e+000

Fig.S3 (A) The intensity-average particle sizes and (B) *Z*eta potentials of PTX-SAL in the dark environment at 4 ± 2 ℃ for 15 days. (C) The drug leakage ratios of PTX in the dark environment at 4 ± 2 ℃ for 15 days. The data are represented as mean ± SD, n = 3.