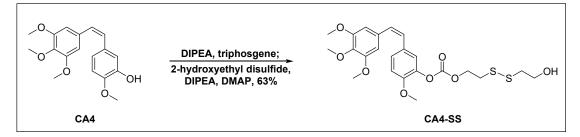
Electronic Supporting Information

for

An activatable, carrier-free, triple-combination nanomedicine for

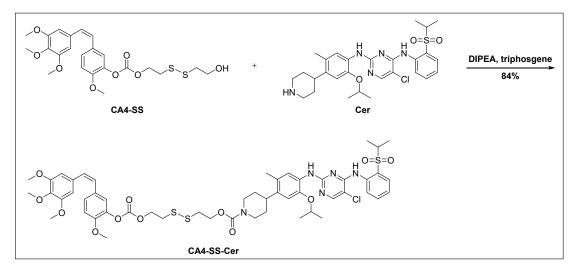
ALK/EGFR-mutant non-small cell lung cancer high-permeable

targeted chemotherapy



To a stirred solution of CA4 (63.2 mg, 0.2 mmol, 1.0 equiv) in dry dichloromethane (DCM, 40 mL) was added tiphosgene (BTC, 1.188 g, 4.0 mmol, 20.0 equiv) at 0 °C. A solution of N,N-diisopropylethylamine (DIPEA, 0.99 mL, 6.0 mmol, 30.0 equiv) in dry DCM (6.0 mL) was dropwise added into the CA4 solution at 0 °C for 5 min and then allowed to warm up to room temperature. The reaction was stirred for 3.0 hours at room temperature under N₂ environment until the completion of the reaction. The mixture was concentrated under reduced pressure afforded the crude product (yellow solid). The crude product was directly used for next step.

The residue crude product was diluted with DCM (10 mL), and then dropped into an dry tetrahydrofuran (THF, 20mL) solution, containing triethylamine (0.82 mL, 0.78 mmol), 2-hydroxyethyl disulfide (154.0 mg, 1.0 mmol, 5.0 equiv), and 4dimethylaminopyridine (DMAP, 14.7 mg, 0.12 mmol, 0.6 equiv) at -10 °C for 10 min and then allowed to warm up to room temperature. The mixture was stirred for another 20.0 hours at room temperature under N₂ environment, and then quenched with water. After evaporated under reduced pressure, and then re-dissolved with DCM, the combined extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified by silica gel column chromatography (petroleum ether / ethyl acetate = 1:2) to give CA4 (62.6 mg, 63.1%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃, ppm): δ 7.15 (dd, J = 2.0, 8.0 Hz, 1H), 7.10 (d, J = 2.0 Hz, 1H), 6.87 (d, J = 8.0 Hz, 1H), 6.49 (s, 2H), 6.44 (s, 2H), 4.47 (t, J = 7.0 Hz, 2H), 3.86 (t, J = 8.0 Hz, 2H), 3.84 (s, 3H), 3.83 (s, 3H), 3.71 (s, 6H), 3.03 (t, J = 7.0 Hz, 2H), 2.88 (t, J = 6.0 Hz, 2H), 2.32 (s, 1H). ¹³C NMR (125 MHz, CDCl₃, ppm): δ 153.03, 153.00, 150.19, 137.18, 132.38, 130.10, 129.77, 128.36, 128.11, 122.69, 112.21, 105.86, 66.51, 60.92, 60.27, 56.04, 55.94, 41.53, 36.71.



To a stirred solution of CA4-SS (52.0 mg, 0.105 mmol, 1.0 equiv) in dry DCM (25 mL) was added tiphosgene (BTC, 622 mg, 2.10 mmol, 20.0 equiv) at 0 °C. The DIPEA (0.52 mL, 3.15 mmol, 30.0 equiv) was dropwise added into the CA4-SS solution at -10 °C for 5 min and then allowed to warm up to room temperature. The reaction was stirred for 4.5 hours at room temperature under N_2 environment until the completion of the reaction. The mixture was concentrated under reduced pressure afforded the crude product (yellow solid).

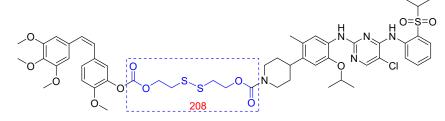
The residue crude product was diluted with DCM (12 mL), and then dropped into an dry DCM (30mL) solution, containing DIPEA (0.020 mL), and Cer (58.5 mg, 0.105 mmol, 1.0 equiv) at -10 °C for 10 min and then allowed to warm up to room temperature. The mixture was stirred for another 20.0 hours at room temperature under N₂ environment, and then quenched with water. After evaporated under reduced pressure, and then re-dissolved with DCM, the combined extracts were washed with brine, dried over Na₂SO₄ and concentrated. The crude product was purified by silica gel column chromatography (petroleum ether / ethyl acetate = 1:1) to give CA4-SS-Cer (95.1 mg, 83.9 %) as white solid. R_f 0.55 (petroleum ether / ethyl acetate = 1:1); ¹H NMR (500 MHz, CDCl₃, ppm): δ 9.61 (s, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 8.13 (s, 1H), 7.96 (s, 1H), 7.93 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.78 (s, 1H), 7.63-7.58 (m, 1H), 7.27 (t, *J* = 8.0 Hz, 1H), 7.14 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.10 (d, *J* = 2.0 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 6.71 (s, 1H), 6.49 (s, 2H), 6.46 (s, 2H), 4.58-4.50 (m, 1H), 4.47 (t, *J* = 7.0 Hz, 1H), 4.44-4.20 (m, 4H), 3.84 (s, 3H), 3.83 (s, 3H), 3.70 (s, 6H), 3.30-3.20 (m, 1H), 3.04-2.97 (m, 4H), 2.95-2.78 (m, 3H), 2.17 (s, 3H), 1.82-1.50 (m, 4H), 1.36 (d, J = 7.0 Hz, 6H), 1.32 (d, J = 6.0 Hz, 6H). HRMS (ESI⁺): m/z calcd for $[C_{52}H_{63}CIN_5O_{12}S_3]$ 1080.3324, found at 1080.3332.

Preparation of CA4-SS-Cer NPs and UA@CA4-SS-Cer NPs

The nanoparticles were prepared by the nanoprecipitation method. Briefly, CA4-SS-Cer (0.54 mg) was dissolved in EtOH (1.1 mL) and then added dropwise into deionized water (9.9 mL) under vigorous stirring at room temperature for 30 min. After that, the solution was dialyzed against distilled water to remove EtOH and un-self-assembled molecules.

As for UA@CA4-SS-Cer NPs, the mixture of CA4-SS-Cer (0.54 mg) and UA (0.23 mg) was dissolved in EtOH, and then the remaining procedure was similar to the preparation of UA@CA4-SS-Cer NPs.

Drug loading efficiency (DLE) was calculated using the following formula: DLE (%) = Weight of encapsulated drug/Weight of the whole nanoparticles \times 100%.



CA4-SS-Cer Mol. Wt.: 1079 CA4-SS-Cer NPs: DLE (%) = (1079-208)/1079 = 80.7% UA@CA4-SS-Cer NPs: DLE (%) > 80.7%

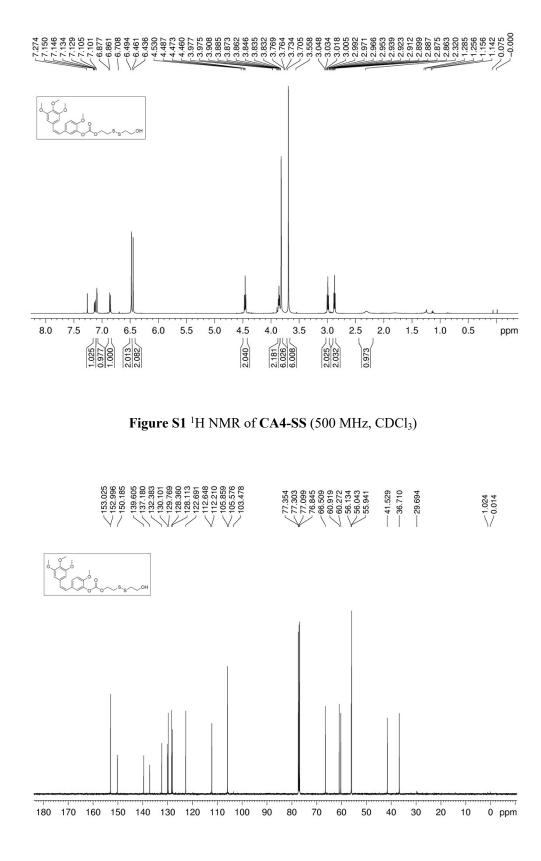


Figure S2 ¹³C NMR of CA4-SS (125 MHz, CDCl₃)

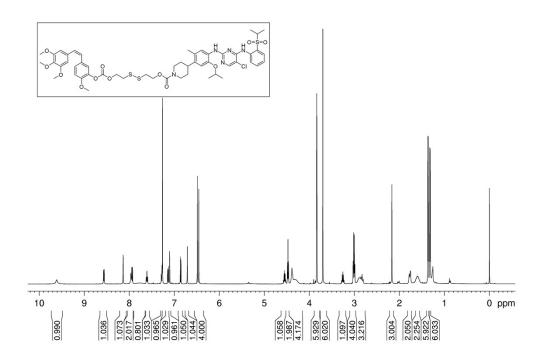


Figure S3 ¹H NMR of CA4-SS-Cer (500 MHz, CDCl₃)

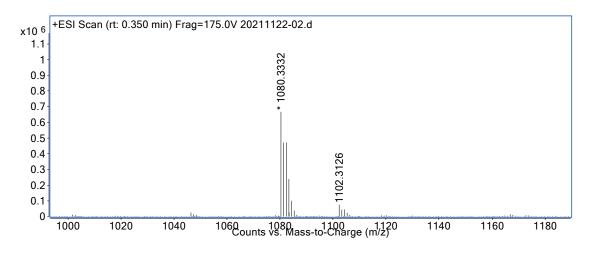


Figure S4 ESI-MS (m/z) $[M+H]^+$ of CA4-SS-Cer

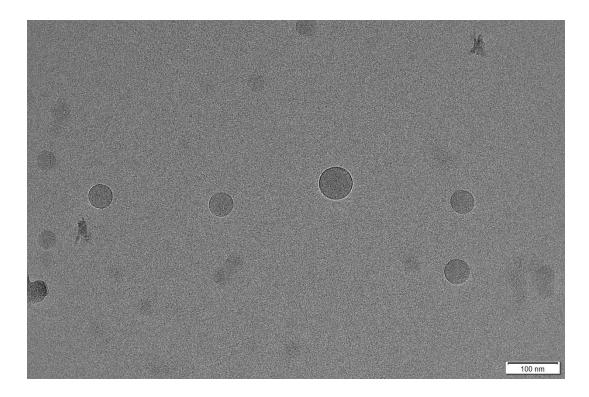


Figure S5 The TEM image of CA4-SS-Cer NPs

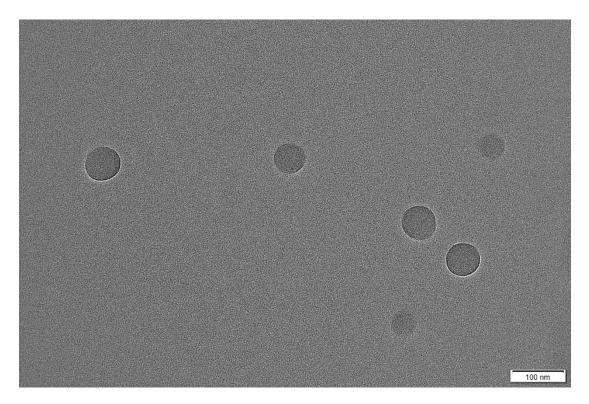


Figure S6 The TEM image of UA@CA4-SS-Cer NPs

Size Distribution Report by Intensity

v2.2



Sample Details Sample Name: 1 SOP Name: mansettings.nano General Notes: File Name: 2021-05-12.dts Dispersant Name: Water Record Number: 10 Dispersant RI: 1.330 Material RI: 1.59 Viscosity (cP): 0.8872 Material Absorbtion: 0.010 Measurement Date and Time: 2021年10月26日 15:49:55 System Temperature (°C): 25.0 Duration Used (s): 70 Count Rate (kcps): 181.2 Measurement Position (mm): 4.65 Cell Description: Disposable sizing cuvette Attenuator: 9 Results % Intensity: St Dev (d.n... Size (d.n... 28.73 78.13 100.0 Z-Average (d.nm): 68.76 Peak 1: 0.000 Pdl: 0.115 0.000 0.0 Peak 2: Intercept: 0.953 Peak 3: 0.000 0.0 0.000 Result quality Good Size Distribution by Intensity 161 14 Intensity (Percent) 12 10 8 6 4 2 0 0.1 1 10 100 1000 10000 Size (d.nm)

Figure S7.1 The diameter distribution of CA4-SS-Cer NPs in aqueous solution.

F

Record 10: 1



Sample Details Sample Name: 2 SOP Name: mansettings.nano General Notes: File Name: 2021-05-12.dts Dispersant Name: Water Record Number: 11 Dispersant RI: 1.330 Material RI: 1.59 Viscosity (cP): 0.8872 Material Absorbtion: 0.010 Measurement Date and Time: 2021年10月26日 15:52:28 System Temperature (°C): 25.0 Duration Used (s): 70 Count Rate (kcps): 182.6 Measurement Position (mm): 4.65 Cell Description: Disposable sizing cuvette Attenuator: 9 Results Size (d.n... % Intensity: St Dev (d.n... 75.52 100.0 25.19 Z-Average (d.nm): 68.97 Peak 1: Pdl: 0.119 Peak 2: 0.000 0.0 0.000 0.000 0.000 0.0 Intercept: 0.951 Peak 3: Result quality Good Size Distribution by Intensity 20 Intensity (Percent) 15 10 5 0 0.1 1 10 100 1000 10000 Size (d.nm) Record 11: 2

Figure S7.2 The diameter distribution of CA4-SS-Cer NPs in aqueous solution.



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manootango.na	00			
2021-05-12.dts		Dispersant Na	me: Water	
12		Dispersant	t RI: 1.330	
1.59		Viscosity (cP): 0.8872	
0.010	Measurement Date and Time: 2021年10月26日			
25.0		Duration Used	(s): 70	
182.2	Measurer	ment Position (m	nm): 4.65	
Disposable sizin	ntor: 9	9		
		Size (d.n	% Intensity:	St Dev (d.n
68.30	Peak 1:	78.05	100.0	30.06
0.127	Peak 2:	0.000	0.0	0.000
0.951	Peak 3:	0.000	0.0	0.000
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	68.30 0.127 0.951 Good	12 1.59 0.010 Measurer 25.0 182.2 Measurer Disposable sizing cuvette 68.30 Peak 1: 0.127 Peak 2: 0.951 Peak 3: Good Size Distribution 1 10 Size	12 Dispersant 1.59 Viscosity (0.010 Measurement Date and Till 25.0 Duration Used 182.2 Measurement Position (m Disposable sizing cuvette Attenua 68.30 Peak 1: 78.05 0.127 Peak 2: 0.000 0.951 Peak 3: 0.000 Good Size Distribution by Intensity	12 Dispersant RI: 1.330 1.59 Viscosity (cP): 0.8872 0.010 Measurement Date and Time: 2021年10月 25.0 Duration Used (s): 70 182.2 Measurement Position (mm): 4.65 Disposable sizing cuvette Attenuator: 9 Size (d.n % Intensity: 68.30 Peak 1: 78.05 100.0 0.127 Peak 2: 0.000 0.0 0.951 Peak 3: 0.000 0.0 Good

Figure S7.3 The diameter distribution of CA4-SS-Cer NPs in aqueous solution.

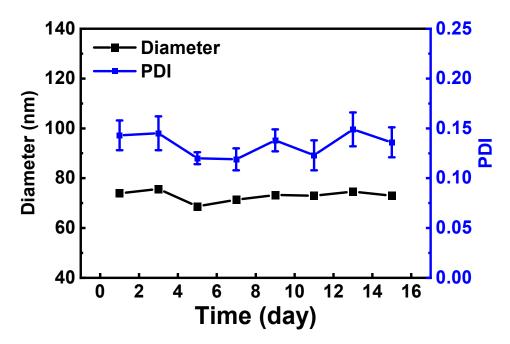


Figure S8 The change of average diameter and PDI of CA4-SS-Cer NPs over time.

Size Distribution Report by Intensity



ample Details						
Sample Name:	2					
SOP Name:	mansettings.n	ano				
General Notes:						
File Name:	1023-0.05 乙酮	∛-3.dts	Dispersant Na	me: Water		
Record Number:	10		Dispersan	t RI: 1.330		
Material RI:	1.59		Viscosity (cP): 0.8872		
Material Absorbtion:	0.010	Measurer	ime: 2021年10月	2021年10月28日 11:57:18		
System						
Temperature (°C):	25.0		Duration Used	(s): 70		
Count Rate (kcps):	222.2	Measurer	ment Position (n	nm): 4.65		
Cell Description:	Disposable siz	ing cuvette	Attenua	ator: 8		
Results						
			Size (d.n	% Intensity:	St Dev (d.n	
Z-Average (d.nm):	91.93	Peak 1:	105.9	100.0	41.50	
Pdl:	0.122	Peak 2:	0.000	0.0	0.000	
Intercept:	0.954	Peak 3:	0.000	0.0	0.000	
Result quality	Good					
	s	Size Distributio	n by Intensity			
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0.1	1	10 Size	100 (d.nm)	1000	10000	
1						

Figure S9.1 The diameter distribution of UA@CA4-SS-Cer NPs in aqueous solution.





Sample Details							
Sample Name:	2						
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General Notes:	mansettings.nano						
General Notes:							
File Name:	1023-0.05 乙醇-	3.dts	Dispersant Na	me: Water			
Record Number:	10		Dispersant	t RI: 1.330			
Material RI:	1.59		Viscosity (cP): 0.8872			
Material Absorbtion:	0.010	Measure	ime: 2021年10月	2021年10月28日 11:57:18			
System							
Temperature (°C):	25.0		Duration Used	(s): 70			
Count Rate (kcps):	222.2	Measurer	ment Position (m	1m): 4.65			
Cell Description:	Disposable sizin	ig cuvette	Attenua	ntor: 8			
Results							
			Size (d.n	% Intensity:	St Dev (d.n		
Z-Average (d.nm):	91.93	Peak 1:	105.9	100.0	41.50		
Pdl:	0.122	Peak 2:	0.000	0.0	0.000		
Intercept:	0.954	Peak 3:	0.000	0.0	0.000		
Result quality	Good						
	Siz	ze Distributio	n by Intensity				
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0.1	83		(d.nm)	1000	10000		
			266 253 277				

Figure S9.2 The diameter distribution of UA@CA4-SS-Cer NPs in aqueous solution.



ample Details						
Sample Name:	3					
SOP Name:	mansettings.nano					
General Notes:						
File Name:	1023-0.05 乙醇-	3.dts	Dispersant Nam	e: Water		
Record Number:	11					
Material RI:	1.59		Viscosity (cP): 0.8872		
Material Absorbtion:	0.010	Measure	e: 2021年10月	2021年10月28日 11:59:41		
ystem						
Temperature (°C):	25.0 Duration Used (s): 70					
Count Rate (kcps):	221.2	221.2 Measurement Position (mm):				
Cell Description:	Disposable sizir	ng cuvette	Attenuato	ttenuator: 8		
esults						
			Size (d.n	% Intensity:	St Dev (d.n	
Z-Average (d.nm):	91.42	Peak 1:	103.3	100.0	39.28	
Pdl:	0.134	Peak 2:	0.000	0.0	0.000	
Intercept:	0.953	Peak 3:	0.000	0.0	0.000	
Result quality	Good					
	Si	ze Distributio	on by Intensity			
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0.1	1	10 Size	100 (d.nm)	1000	10000	
	ſ	Pe	cord 11: 3			

Figure S9.3 The diameter distribution of UA@CA4-SS-Cer NPs in aqueous solution.

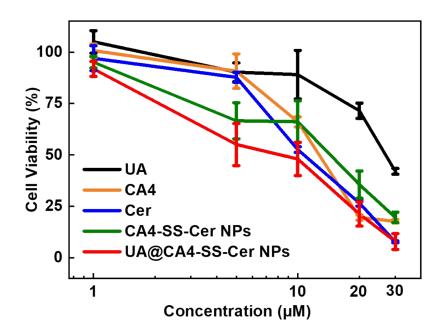


Figure S10 A549 cell viabilities assay (MTT test).



UA@CA4-SS-Cer NPs

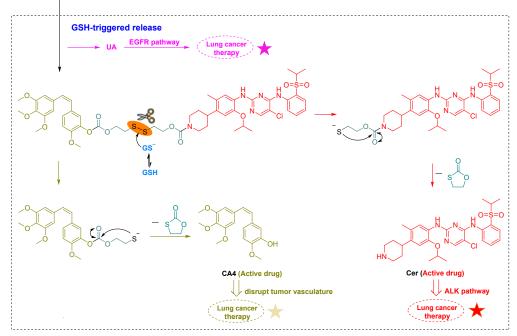


Figure S11 The proposed mechanism of UA@CA4-SS-Cer NPs for the triple-combination deeppenetrating therapy.