A novel phosphoester-based cationic co-polymer nanocarrier delivers chimeric antigen receptor plasmid and exhibits antitumor effect

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Figure S1. The cytotoxicity assay of mPEG-bPEI-PEBP in Jurkat and K562 cells. The results indicate that 200 μ g/mL of mPEG-bPEI-PEBP leads to weak cytotoxicity to Jurkat cells and K562 cells.



Figure S2. The ³¹P NMR characterization of the synthesized 2-ethylbutyl phospholane; a single peak was detected at δ -1.13.



Figure S3. Reactions of synthesis of alkyne-PEBP and ¹H NMR characterization of the product. The corresponding characteristic peaks and integration values are presented. TEA, triethylamine; THF, tetrahydrofuran; TBD, 1,5,7-triazabicyclo[4.4.0]dec-5-ene; DCM, dichloromethane.



Figure S4. The synthesized mPEG(5k)-bPEI(2k)-PEBP was charaterized by ¹H NMR. The corresponding characteristic peaks and integration values are presented. The inset represents a TEM image of co-polymer self-assembled in an aqueous solution (scale bar=200 nm). TMS, tetramethylsilane.



Figure S5. Formation of a complex between the co-polymer and DNA was characterized by agarose gel electrophoresis. Aliquots of 1 (lane 2), 0.5 (lane 3), 0.25 (lane 4), 0.125 (lane 5), 0.063 (lane 6), 0.031 (lane 7), and 0.016 (lane 8) mg/mL mPEG-bPEI-PEBP were mixed with 20 μ g/mL CAR plasmids in an aqueous solution and subjected to electrophoresis in 1% agarose gel. Lane 1, DNA molecular weight marker.



10

0-

12h

Figure S6. Fluorescent images illustrating the transfection efficiency of the mPEG-bPEI-PEBP copolymer and lipofectamine 2000 (Lipo2000) at 12 and 72 h after transfection. A, the co-polymer group at 12 h; B, the Lipo2000 group at 12 h; C, the co-polymer group at 72 h; D, the Lipo2000 group at 72 h; E, Quantitative results. Data are expressed as mean \pm sd. **P < 0.01; NS, not significant.

72h

Time



Figure S7. The standard curves of IFN- $\!\gamma$ and IL-2 obtained by the ELISA method.