Detachable PEG Masking of Checkpoint Antibodies for Immune-Selective Safety and Tumor-Specific Activation

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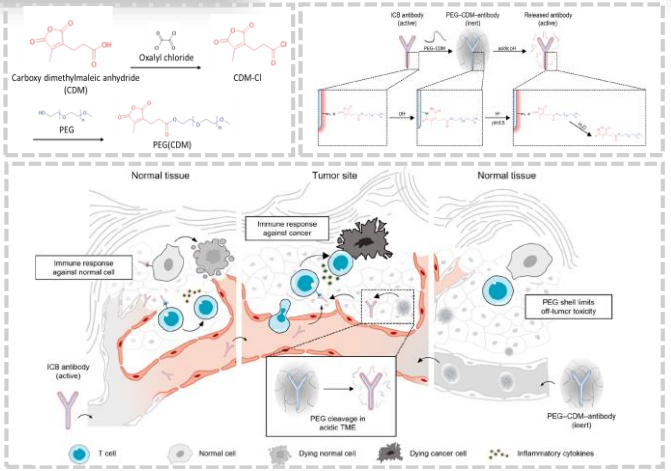
Abstract

Immune checkpoint inhibitors (ICIs) show limited clinical benefit and often cause **immune-related adverse events (irAEs).** We developed **PEG-masked ICIs** via a **pH-responsive linker** that remain inert in normal tissues but activate in the acidic tumor microenvironment. This approach enhanced anti-tumor immunity while reducing irAEs in murine models, offering a safer and more effective alternative to conventional ICIs.

Objective



This study aims to engineer **pH-responsive PEGylated ICIs** via a **CDM linker** to achieve tumor-specific activation while reducing off-target effects and immune-related adverse events (irAEs)

Characteristics

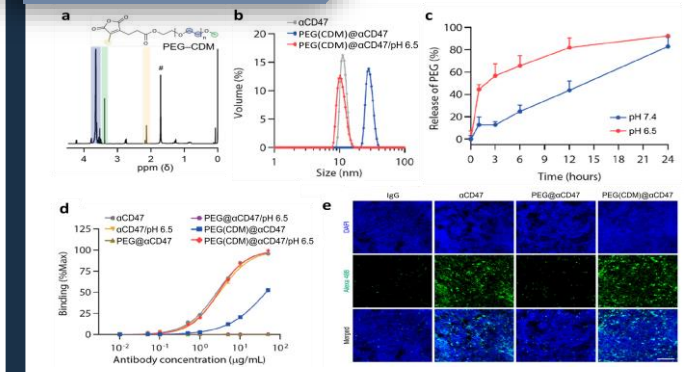


Figure 1. (a) ^{1}HNMR analysis. (b) Size distribution of αCD47, PEG(CDM)@αCD47 and preincubated PEG(CDM)@αCD47 at pH 6.5. (c) Time dependent PEG release from PEG(CDM)@CD47 at pH 7.4 and pH 6.5. (d) Dose dependent binding behaviors of αCD47, PEG@αCD47, PEG(CDM)@αCD47 at different pH. (e) Immunofluorescence microscopic images showing the distribution of unmodified αCD47 and modified αCD47 in tumor tissues.

Conclusion

- The masking strategy preserves the structure and efficacy of antibodies regardless of type.
- Masked antibodies ameliorate irAEs like anemia and colitis.

pH-responsive PEG-masked checkpoint antibodies selectively restore immune activity in the tumor microenvironment, offering a feasible strategy to maintain antitumor efficacy while minimizing immune-related adverse events, a major limitation of ICB-based immunotherapy.

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Results

1. Prevention of αCD47-induced anemia

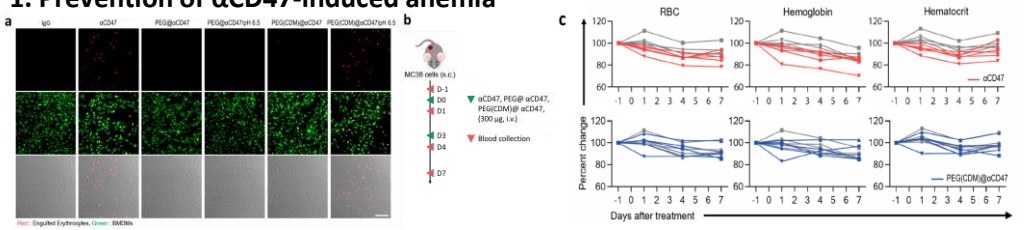

Figure2. (a) CMFDA-labeled BMDMs were cocultured with pHrodo-labeled erythrocytes for 2h in the presence of samples and analyzed by confocal microscopy. (b) Each sample were intravenously administered to the MC38 tumor-bearing mice. (c) RBC, hemoglobin and hematocrit were determined by a hematology analyzer. (n=5)

2. Prevention of αPD1 and αCTLA-4-induced colitis

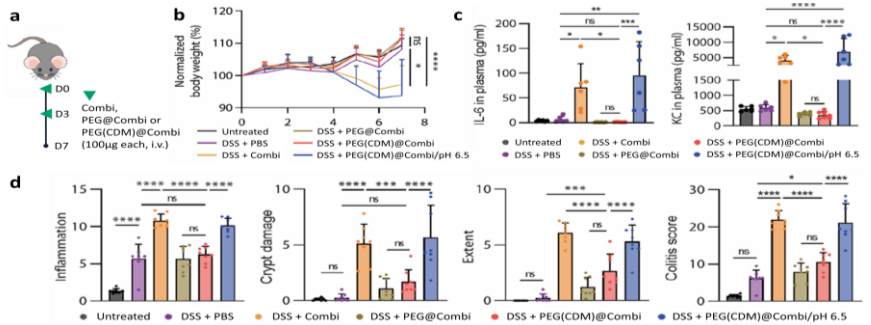

Figure3. (a) Each sample were intravenously administered to the C57/BL6 with DSS-induced colitis. (b) Body weight reduction as a representative marker of colitis severity in mice. (n=8) (c) Inflammatory cytokine IL-6 and KC level in plasma were quantified by ELISA. (d) Colitis scoring for inflammation, crypt damage, extent, and overall colitis from H&E stained colonic tissue sections. (**P < 0.0332, ***P < 0.0002, ****P < 0.0001)

3. Preservation of antitumor efficacy of ICB antibodies by PEG(CDM) conjugation

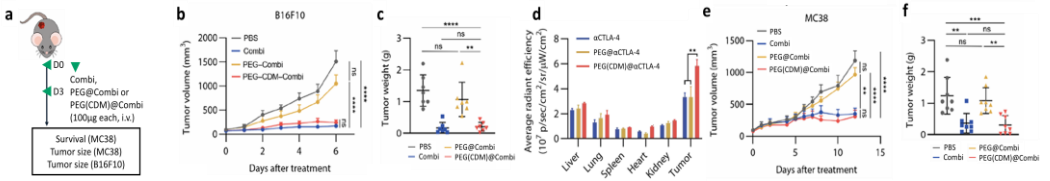

Figure4. (a) Each sample were intravenously administered to the MC38 or B16F10 tumor-bearing C57/BL6 mice. (b) MC38 tumor growth after treatment. (n=8) (c) MC38 tumor weight after treatment. (d) Average radiant efficiency of αCTLA-4, PEG@αCTLA-4, and PEG(CDM)@αCTLA-4 in major organs and tumors at 24 h. (e) B16F10 tumor growth after treatment. (n=7) (f) B16F10 tumor weight after treatment. (**P < 0.0021, ***P < 0.0002, ****P < 0.0001)