Engineering Self-Assembled Protein Nanobarrels for Use as Neoantigen-based Cancer Vaccine

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Cancer vaccine is a promising cancer immunotherapy with sustained immune surveillance and less side effect of non-specific targeting. However, insufficient immunogenicity limits its potential to be an epoch-making cancer therapy. Here, we report a cancer nanovaccine with highly immunogenic protein-based carrier and concatenating neoantigens. BP26, an outer membrane protein of zoonotic bacteria *Brucella*, self-assembles into symmetric hollow barrel-like nanostructure in hexadecamer. We genetically engineered BP26 to display tandem repeats of MHC class II-restricted melanoma neoantigen M30 (BP26-M30 nanobarrels) and the final construct was in size of 10-30nm, appropriate for lymph node drainage. Immunization with BP26-M30 nanobarrels significantly inhibited tumor growth in B16-F10 melanoma-bearing mice, and the effect further enhanced using toll-like receptor 9 (TLR9) agonist, CpG oligodeoxynucleotide (ODN). Also, *ex vivo* splenocyte restimulation showed that BP26-M30 nanobarrels with CpG ODN induced antigen-specific $CD4^+$ T cell response with considerable expression of pro-inflammatory cytokines, like interferon (IFN)-γ. These findings suggest that BP26 nanobarrels is an appropriate platform for cancer nanovaccine which complements low immunogenicity of cancer vaccine to defeat highly aggressive tumors.

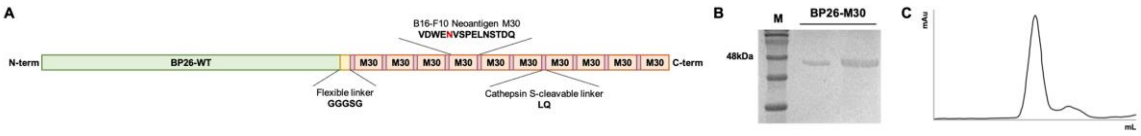

Figure 1. Construction and expression of multivalent B16-F10 neoantigen M30-displaying BP26. (A) Schematic representation of M30-displaying BP26 design. (B) SDS-PAGE gel image of BP26-M30. (C) Size-exclusion chromatography of BP26-M30.

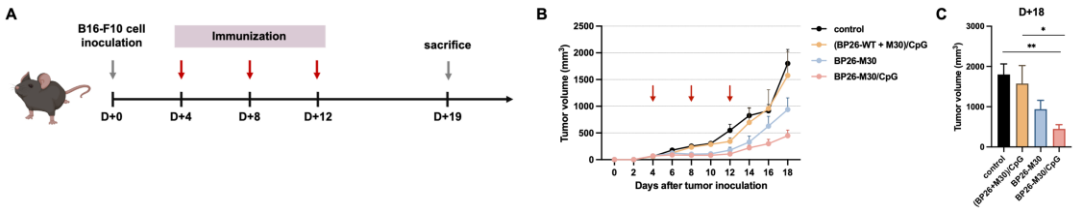

Figure 2. Antitumor efficacy of BP26-M30 nanobarrel in B16-F10 tumor-bearing mice. (A) Immunization schedule of mice. (B) Average tumor growth curve of B16-F10. (C) Final tumor volume of mice, 18 days after B16-F10 cancer cell inoculation.

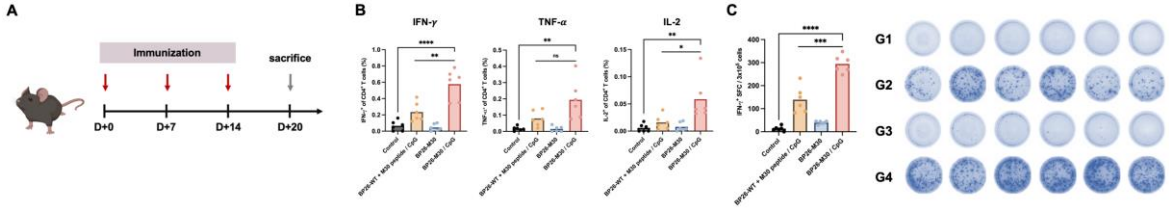

Figure 3. Antigen-specific T cell response by BP26-M30 cancer vaccine immunization. (A) Proportion of CD4+ T cells with high expression of pro-inflammatory cytokine after restimulation. (C) IFN-γ spot forming cells after restimulation.