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ORAL ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

The BCMA-Targeted Fourth-Generation CAR-T Cells Secreting CST6 Against Multiple Myeloma and Suppresses Osteolytic Lesions

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651 Multiple Myeloma and Plasma Cell Dyscrasias: Basic and Translational

Introduction: Multiple myeloma (MM) is characterized by osteolytic lesions, and our previous research has shown that cystatin E/M (CST6) effectively mitigates MM bone disease by inhibiting osteoclast differentiation. However, the therapeutic application of recombinant CST6 protein faces challenges due to its short serum half-life in mouse models, necessitating maintenance of constantly elevated CST6 concentrations for optimal efficacy. In this study, we explore the potential of utilizing fourth-generation B cell maturation antigen (BCMA)-specific chimeric antigen receptor (CAR)-T cell therapies to address this limitation. By leveraging the secretion capabilities of CST6, we engineered BCMA-CST6-CAR-T cells to precisely target nascent focal lesions. Our results demonstrate that these engineered CAR-T cells effectively target MM cells and concurrently suppress osteolytic lesions, presenting a promising approach for combating MM and its associated bone pathology.

Methods: The BCMA-CST6-CAR vector comprises BCMA-scFv, a 4-1BB co-activation domain, and CD3ζ. To facilitate separate expression, a P2A self-cleaving peptide was introduced between CAR and CST6. CAR-T cells were co-cultured with MM1.S cells at a 5:1 ratio for 24 hours. To assess in vivo anti-tumor activity and anti-bone resorption of BCMA-CST6-CAR-T cells, we engrafted luciferase-expressing MM1.S cells into NSG mice. On day 7, mice with established MM received CAR-T cell infusions. At day 35, the mice were euthanized, and we analyzed serum CST6 concentrations using ELISA. Additionally, we collected mouse tibiae to perform μCT scans and TRAP staining to evaluate bone resorption.

Results: The co-culturing of BCMA-CST6-CAR-T cells with MM1.S cells resulted in a remarkable 77.2% lysis of MM1.S cells after 24 hours, with no significant difference in killing observed between the BCMA-CAR-T and BCMA-CST6-CAR-T groups. However, T cell activation marker CD69 exhibited a significant increase in the BCMA-CST6-CAR-T group (70.9%) compared to the MOCK-CAR-T group (9.2%). In the MM-bearing mouse model, bioluminescence imaging revealed that BCMA-CST6-CAR-T cells exhibited excellent antitumor activity, leading to near-complete tumor clearance by day 21. Notably, both BCMA-CST6-CAR-T and BCMA-CAR-T cells showed similar effects in inhibiting tumor growth. The in vivo serum concentration of CST6 was significantly higher in the BCMA-CST6-CAR-T group (938.4 ng/ml) compared to the MOCK group (60.9 ng/ml) and BCMA-CAR-T group (64.2 ng/ml) ($P < 0.001$), highlighting the robust secretion of CST6 protein by BCMA-CST6-CAR-T cells. Moreover, μCT images of mouse tibiae demonstrated that BCMA-CST6-CAR-T cells effectively suppressed osteolytic lesions in MM-bearing mice. This finding was further corroborated by TRAP staining of mouse tibia sections, which showed a significant reduction in osteoclast numbers and the proportion of bone surface occupied by osteoclasts in the presence of BCMA-CST6-CAR-T cells.

Conclusion: These results underscore the potent antitumor and anti-bone resorption effects of BCMA-CST6-CAR-T cells and their potential as a promising therapeutic approach for multiple myeloma.