Title : Designing and Engineering BiKEs for Expression In HEK293T cells.

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Major : Biology

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ABSTRACT

Bispecific Killer Engagers (BiKEs) are innovative immunotherapeutic molecules designed to enhance immune cell-mediated cytotoxicity against cancer cells. In this study, a BiKEs construct incorporating anti-GD2 and anti-CD16 was genetically engineered via cloning techniques. Additionally, the anti-GD2 BiKEs was designed to secrete IL-15 to enhance the proliferation of NK cells, referred to as anti-GD2 BiKEs secreting IL-15. The anti-GD2 BiKEs secreting IL-15 were subsequently cloned into a lentiviral vector for stable expression in HEK293T cells. This construct was incorporated with fluorescent and His tags for protein detection and transfection efficiency assessment. Transfection efficiency was confirmed via fluorescence inverted microscopy, while western blot analysis validated protein expression using an anti-His antibody. However, western blot results showed no detection of the Myc-tag, and sequencing analysis revealed discrepancies between the obtained sequence and the expected template. These findings suggest that the cloned protein was not the intended insert, likely due to incorrect sequence integration or unexpected mutations. Consequently, the addition of IL-15 to the construction did not achieve the desired outcome. Despite these setbacks, the study successfully demonstrated robust BiKEs expression, providing a foundation for further optimization and functional validation. Future studies will focus on refining the construct and confirming its functional role in NK cell activation and cytotoxicity assays. This research underscores the

potential of BiKEs based immunotherapy targeting GD2-positive malignancies, such as neuroblastoma and melanoma, paving the way for further preclinical development.