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I.S. Orel¹, P.S. Yurko², N.Ye.Volkova¹

¹ V.N.Karazin Kharkiv National University
4, Svobody Sq., Kharkiv, Ukraine, 61022

² National Scientific Center «Institute of Experimental
and Clinical Veterinary Medicine»
83, Hryhoriia Skovorody Str., Kharkiv, Ukraine, 61023
vanorel2007@gmail.com

COMPREHENSIVE *IN SILICO* DESIGN OF A NEXT-GENERATION ONCOLYTIC HAdV-5 ADENOVIRUS WITH MODIFIED TROPISM, IMMUNE MASKING, AND TUMOR-SPECIFIC REPLICATION

Background/Aim. The use of adenoviruses (HAdV-5) for the treatment of aggressive tumors has limitations due to their natural tropism for healthy cells, the risk of off-target replication, and rapid vector neutralization by antibodies. The aim of this study is to develop an *in silico* model of a HAdV-5 vector that combines detargeting, retargeting to the tumor marker EGFRvIII (Gan, H.K., et al., 2013), identification of sites for immune masking, and conditional replication under the control of the hTERT promoter. **Methods.** Vector design and *in silico* cloning were performed in Benchling. 3D structures were modelled using AlphaFold2, and spatial compatibility was assessed with HDock. **Results.** Specific steps were undertaken to develop an adenoviral model with triple control. Detargeting (Kirby, I., et al., 2000) was achieved by substituting three amino acids (S23A, P24A, Y92A), which served as conditional hooks, on the capsid surface. These were replaced with the neutral amino acid alanine, disrupting binding to the CAR receptor. Integration of an anti-EGFRvIII nanobody (Bagchi, A., et al., 2024) via a standard (Gly₄Ser)₃ linker enabled retargeting to the glioblastoma target, improving spatial compatibility from -285.39 to -330.47 in HDock score. The nanobody recognizes a unique neoepitope (formed upon deletion of exons 2–7 in the EGFR gene) that is absent in wild-type EGFR and the normal human proteome, minimizing off-target effects (Gan, H.K., et al., 2013). An immune masking strategy was developed: N-glycosylation motifs (N-F-S/N-T-S) were introduced into the hypervariable regions (HVR5/HVR7) of the hexon via I333S/Y500S mutations. Our AlphaFold2 model confirmed high structural stability (pLDDT > 90) and surface accessibility of mutated residues, supporting their potential for glycosylation. Docking with antibody 5LDN confirmed that shielding residues 333 and 500 could block antibody access. Replacement of the endogenous E1A promoter with the tumor-specific hTERT promoter enabled selective replication in tumor cells, while *in silico* cloning confirmed correct integration and preserved 5'-UTR integrity. **Study limitations.** Results are based on static docking and require validation by molecular dynamics and cell-based assays. Mutations may represent potential glycosylation sites or targets for alternative immune masking (e.g., PEGylation). **Conclusions.** Consistent steps were used to develop an *in silico* HAdV-5 model with reprogrammed tropism, transcriptional restriction, and defined immune-shielding sites. The design shows promise as a safer gene therapy candidate than early vectors such as ONYX-015.

Keywords: oncolytic viruses, *in silico* design, HAdV-5 adenovirus, tropism modification.

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