P–32; **ENHANCEMENT OF T CELL-MEDIATED AND HUMORAL IMMUNITY OF β-GLUCURONIDASE-BASED DNA VACCINES AGAINST HPV-16 E7 ONCOPROTEIN**

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Background: Therapeutic DNA vaccines against oncogenic infection with human papillomavirus type 16 (HPV-16) are mostly targeted against viral oncoproteins E7 and E6. To adapt the E7 oncoprotein for DNA immunization, we have previously reduced its oncogenicity by modification of the Rb-binding site and enhanced immunogenicity of the modified E7GGG gene by its fusion with the 5' terminus of the gene encoding *E. coli* ß-glucuronidase (GUS) generating E7GGG. GUS. In this study, we attempted to improve immunogenicity of the GUS-based anti-E7 vaccines by increasing the steady-state level of fusion proteins.

Materials and Methods: We fused deletion mutants of E7GGG and codon-optimized E7GGG with the 5' terminus of GUS and unaltered E7GGG with the 3' terminus of GUS. Furthermore, we mutated the initiation codon of the GUS gene in the E7GGG.GUS construct, as GUS alone was produced from this fusion gene.

Results: We have found that only the fusion of E7GGG with the 3' terminus of GUS (GUS.E7GGG) and deletion mutants of E7GGG with the 5' terminus of GUS increased the steady-state level of fusion proteins in transfected human 293T cells. Analysis of immune reactions induced in mice by vaccination via a gene gun showed that the increased steady-state level of fusion proteins resulted in augmented production of E7-specific antibodies, but did not enhance cell-mediated anti-tumor immunity. Finally, we joined the signal sequence of the adenoviral E3 protein with GUS.E7GGG. This modification led to the predominant localization of the fusion protein in the endoplasmic reticulum and enhancement of $CD8^+$ T-cell response while antibody production was reduced.

Conclusions: To conclude, we have found modifications of the E7GGG.GUS fusion gene that augmented either humoral or cell-mediated immune responses.

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