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Development of stealth transgenes for gene therapy : evaluation of cis-acting inhibitors of antigen presentation

Raamsman-Ossevoort, M.

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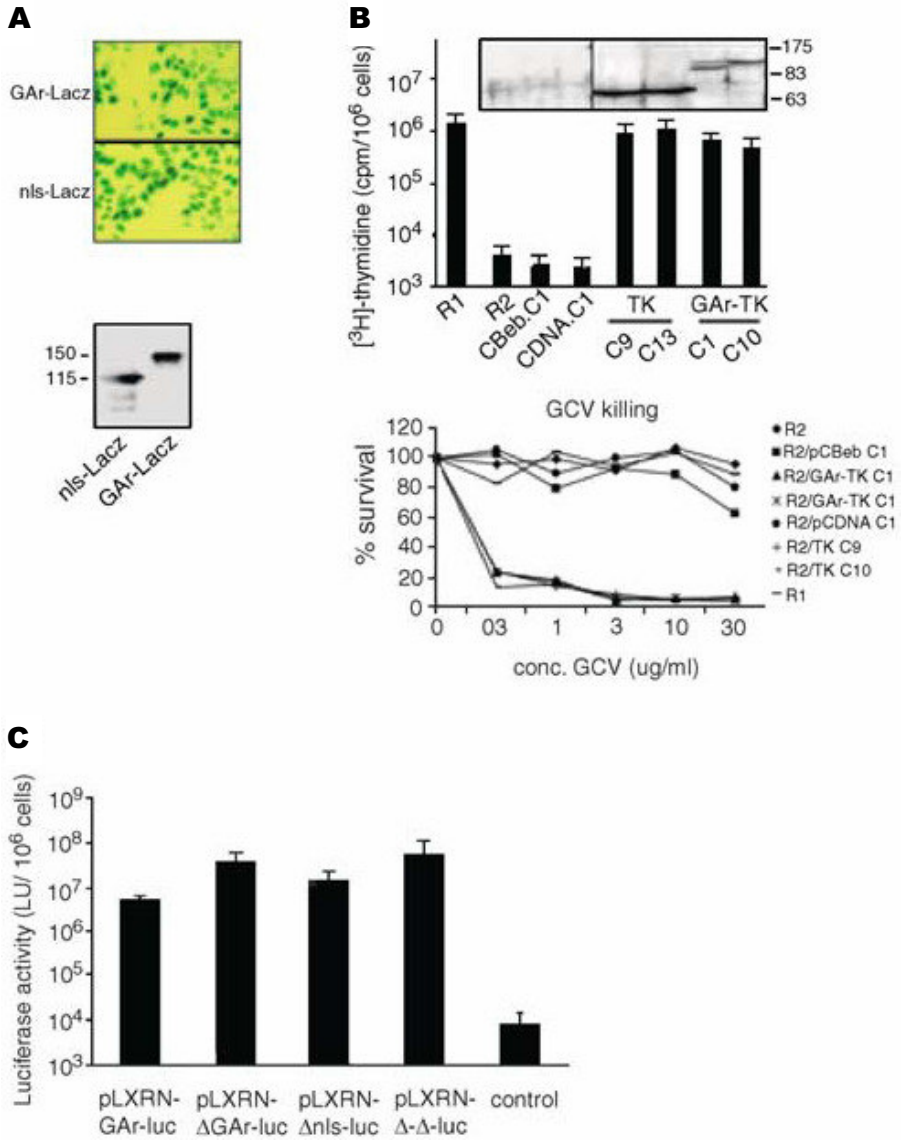
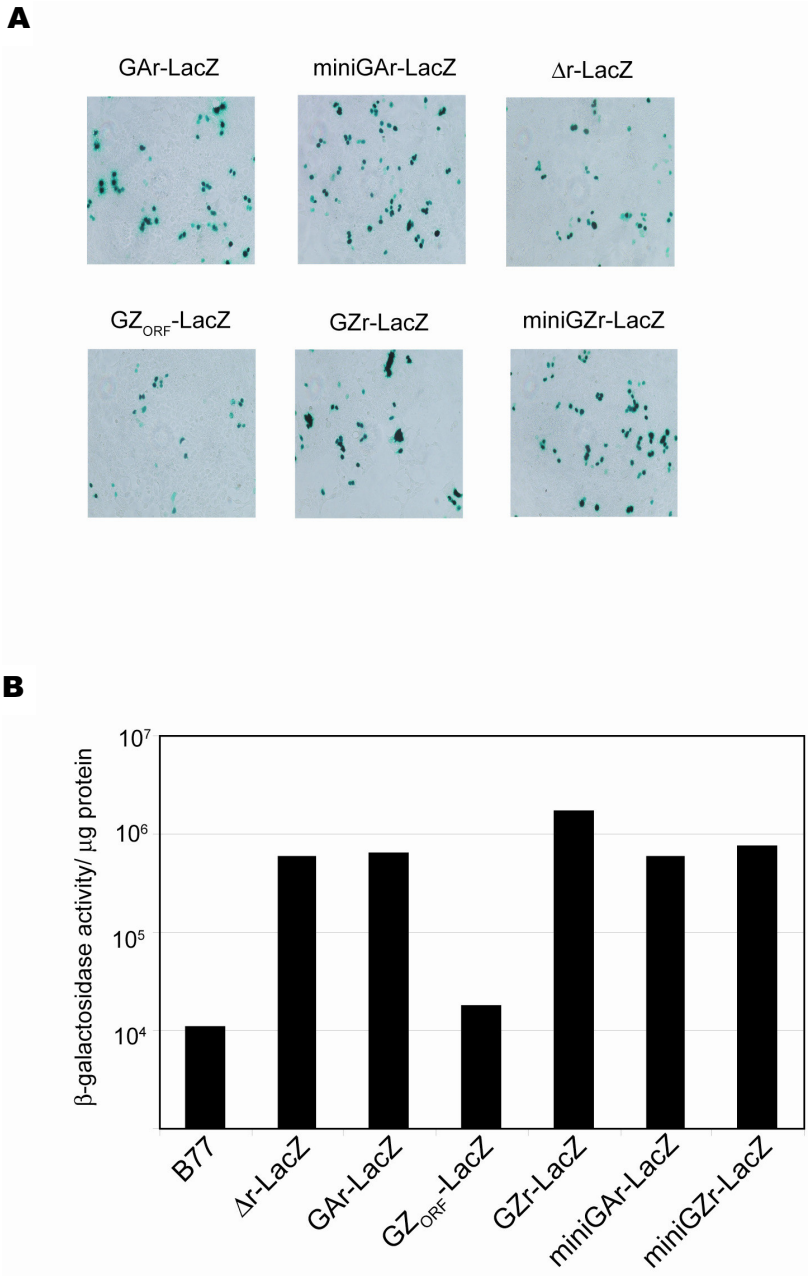
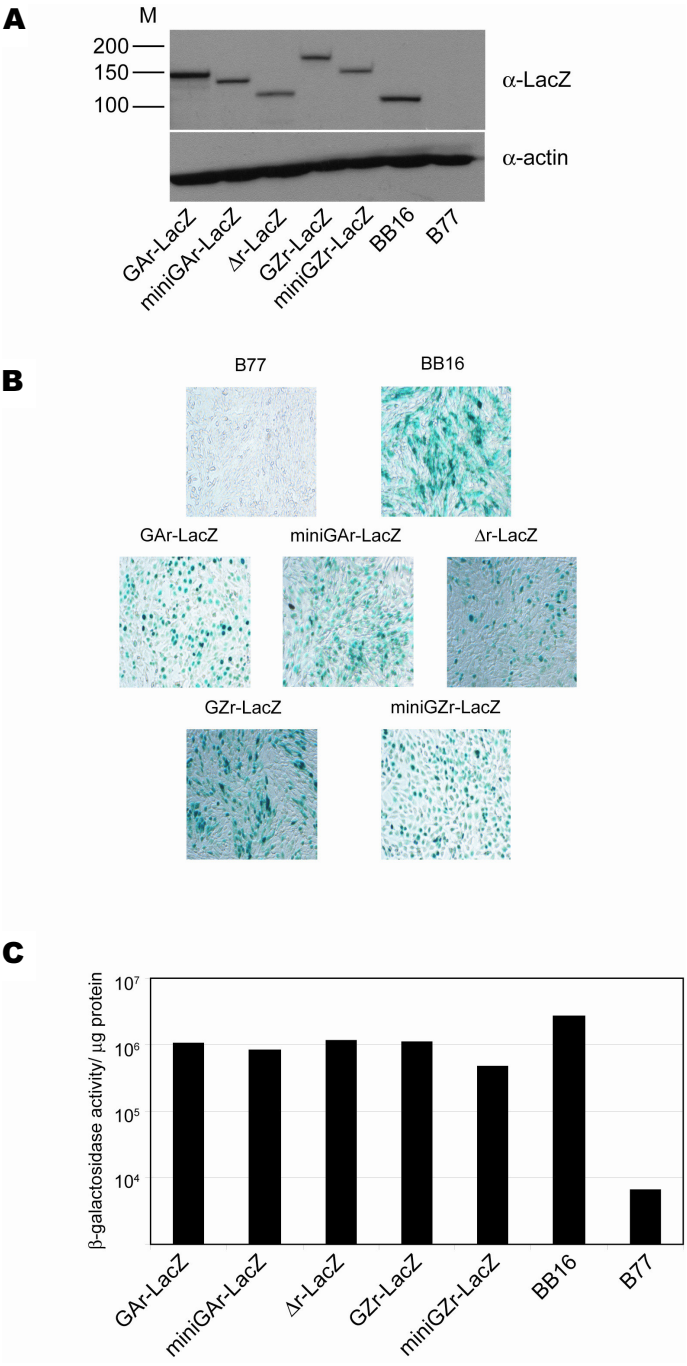
Appendix: color figures



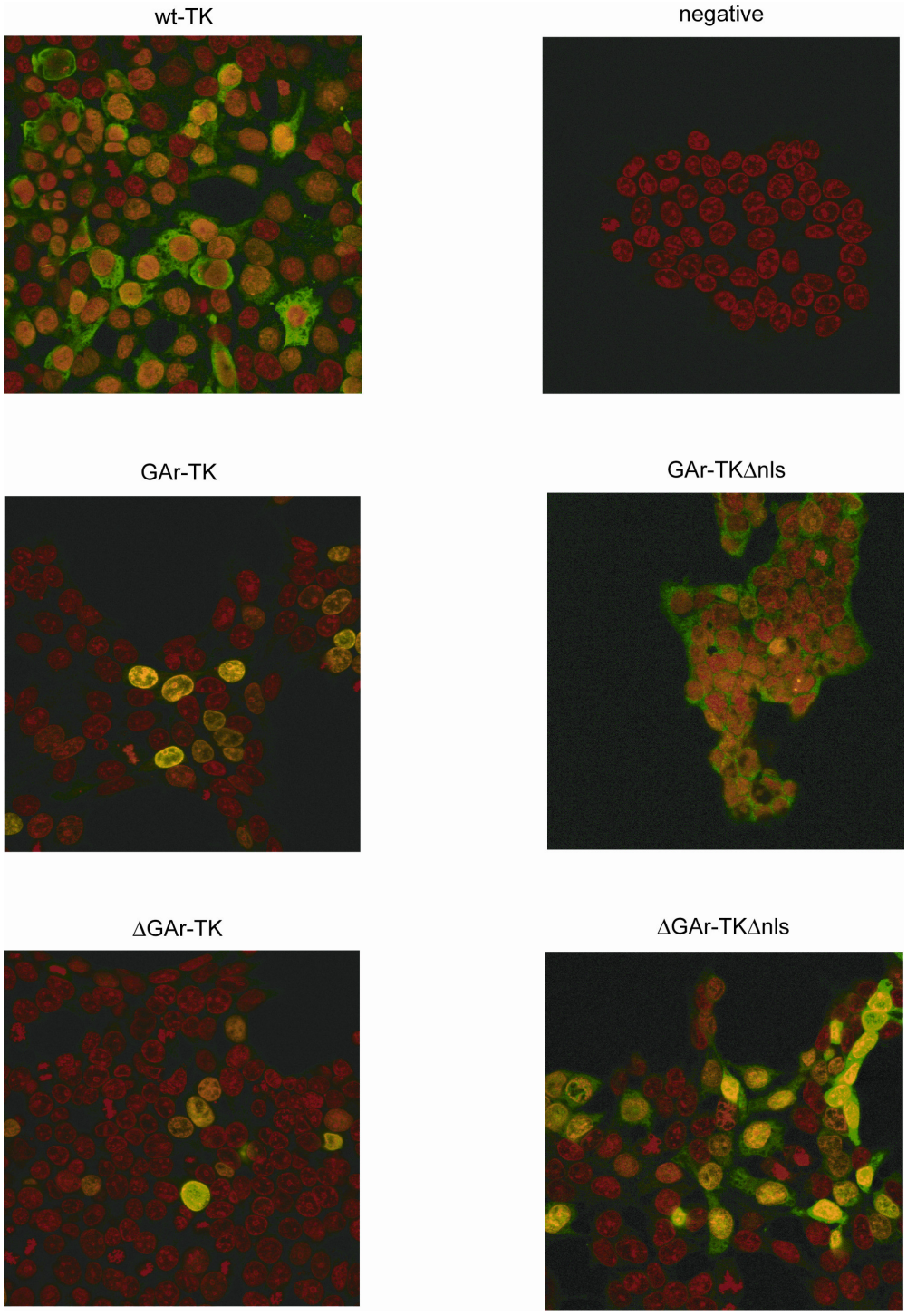
Chapters 2, figure 2. GAr fusion enzymes retain their activity. (a) Activity of GAr-β-galactosidase. Hep2 cells were infected with rAd5-nls-LacZ or rAd5-GAr-LacZ (4 PFU/cell). At 48 h postinfection, the cells were fixed and stained for β-galactosidase activity. In addition, H_{1299} cells grown in 10-cm dishes were infected with rAd5-nlsLacZ or rAd5-GArLacZ with 10 PFU/cell. After 48 h, protein extracts were made and size-fractionated by SDSPAGE, transferred to nitrocellulose membranes and probed with an anti-LacZ mouse monoclonal antibody (Roche, Basel, Switzerland). (b) Activity of GAr-TK. The TK-deficient cell line Rat2 (R2, TK^-) was used to generate stable cell lines containing the various plasmids. CBeb.C1 and CDNA.C1 are G418-resistant Rat2 cells obtained after transfection of the empty pCBeb and pCDNA3.1 plasmids, respectively. GArTK.C1 and C10 are independent G418-resistant clones of Rat2 cells stably expressing the pGAr-TK plasmid. TK.C9 and C13 are two independent G418-resistant Rat2 clones derived by transfection of the plasmid pCDNA-TK. Rat1 (R1) is the TK^+ parental cell line from which Rat2 had been derived. [^{3}H]thymidine incorporation (± s.d.) is represented per 10^6 cells. The inset depicts a Western analysis of the Rat2 clones with a polyclonal goat anti-HSV-TK antibody. The faster-migrating band in clone GAr-TK C1 may result from splicing within the TK coding region.[59] The same cell lines were analyzed for their ganciclovir sensitivity by growing these cells for 48 h in the presence of varying concentrations of ganciclovir. Cell viability was determined with the WST-I colorimetric assay. (c) Activity of GArluciferase. Cultures of 911 cells were transfected with pCBeb (as a negative control), pLXRN-GAr-luc and the GAr- and nls-deletion derivatives. Cells were lysed 18 h post-transfection and the luciferase activity was measured in the lysates. The mean of three experiments is shown, expressed as light units/10^6 cells ± 1 s.d.



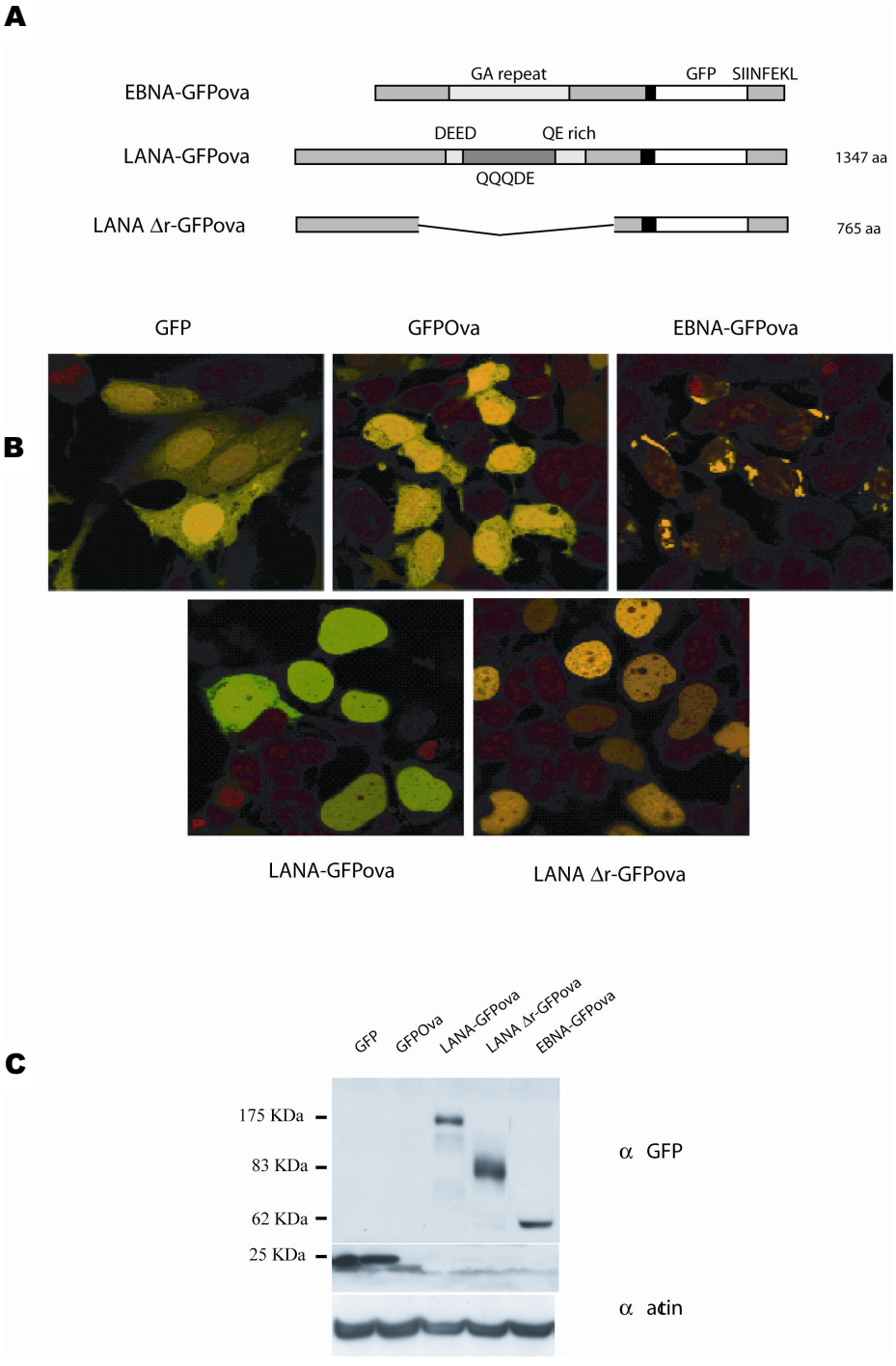
Chapter 3, figure 3. *In-situ* β-galactosidase activity assay (A) and galactolight assay (B) of the transfected 293T cells. A 293T cells were transfected with miniGZr-LacZ, miniGAr-LacZ, GZr-LacZ, GZ_{ORF}-LacZ, GAr-LacZ and Δr-LacZ-encoding plasmids. After 48 hours, the cells were fixed, and stained overnight. (B) 293T cells were transfected with miniGZr-LacZ, miniGAr-LacZ, GZr-LacZ, GZ_{ORF}-LacZ, GAr-LacZ and Δr-LacZ encoding plasmids. After 48 hours, the cells were lysed and galacton was added. The β-galactosidase activity is normalized for the protein concentration in the lysate.



Chapter 3, figure 4. Characterization of the stable B77 cell lines. B77 cells were transfected with plasmids encoding miniGZr-LacZ, miniGAr-LacZ, GZr-LacZ, GAr-LacZ, and Δr-LacZ proteins respectively. After establishing stable cell lines, the cells expressing miniGZr-LacZ, miniGAr-LacZ, GZr-LacZ, GAr-LacZ, and Δr-LacZ, as well as B77 and BB16 cells (B77 cells expressing LacZ) were lysed and protein extracts were size fractionated by SDS-PAGE (A). The western blot was analyzed with a monoclonal mouse anti-LacZ antibody. All proteins migrate at the expected size, and no smaller products are visible. (B) The same cell lines, as well as B77 and BB16 were fixed stained *in-situ*. (C) Stable B77 cell lines expressing the miniGZr-LacZ, miniGAr-LacZ, GZr-LacZ, GAr-LacZ, and Δr-LacZ constructs, and B77 and BB16 cells were lysed and galacton was added. Plotted is the normalized β-galactosidase activity.



Chapter 4, figure 2. Sub-cellular localization of the different TK proteins. 293T cells were transfected with wt-TK, GAr-TK, GAr-TKΔnls, ΔGAr-TK or ΔGAr-TKΔnls. Cells were incubated with rabbit anti-HSV-TK antiserum and a FITC-conjugated goat-anti-rabbit secondary antibody. The nucleus was stained with Propidium Iodide and the cells were analyzed by a confocal laserscan microscopy. Deletion of the nls but not the GAr restores the wt-TK distribution pattern.



Chapters 5, figure 2. GFP fusion proteins. (A) Schematic diagram of pRRL-CMV-EBNA-GFP$_{OVA}$, pRRL CMV-LANA-GFP$_{OVA}$ and pRRL-CMV-LANAΔr-GFP$_{OVA}$. (B) Cellular localization of modified GFP. Confocal microscopy pictures taken 60h after transfection in 911 cells. (C) Western blot analysis of protein extracts from 911 transfected cells expressing GFP / GFP$_{OVA}$ / LANA-GFP$_{OVA}$ / LANAΔr-GFP$_{OVA}$ / EBNA-GFP$_{OVA}$. Anti-GFP antibody is used for the detection, and loading control is provided by anti-Actin.