

[P-65]**Investigation of genomic integration of GX-12, a new anti-HIV DNA vaccine, into host cellular DNA following intramuscular injection in rats.**

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GX-12 is a naked DNA vaccine developed by research team of Dong-A Pharmaceutical Company, Green Cross Company and Genexine for the treatment of HIV infection. It consists of four separate plasmids (pGX10-GE HX, pGX10-dpol JR, pGX10-VN/TV JR, pGX10-hIL-12m), which were constructed by inserting the HIV-1 gag-env, pol, regulatory genes and a human IL-12 mutant gene into pGX10 plasmid vectors. In the present study we examined the potential of genomic integration of GX-12 into host cellular DNA following intramuscular injection in rats using real-time quantitative PCR.

GX-12 was injected into the left anterior tibialis of both male and female SD rat at the dose of 400 μ g/head once a week for four weeks. On day 1, 5, 15, 30 and 45 days after final administration, muscles were harvested and DNA was extracted. After purification of chromosomal DNA by agarose gel electrophoresis, quantitative real-time PCR (RT-PCR) was performed to assay the plasmid integration. Quantitative real-time PCR was performed using the ABI Prism 7900HT sequence detection system (Applied Biosystems) with SYBR green dye as the detection reagent. The primers sequences were 5'-CAGCAGCTGACACAGGACACA-3' and 5'-ACCATTTGCCCTGGATGT-3' for gag-env amplification, 5'-AAGACTTCTGGGAAGTTCAATTAGGAA-3' and 5'-GCATCACCCACATCCAGTACTG-3' for pol amplification, 5'-AGAACTAACAGAGGATAGATGGAACAA-3' and 5'-CACCCATTCTAGAGTGTCCATTCA-3' for vif-nef-tat-vpu amplification, 5'-TGGAGTGCCAGGAGGACAGT-3' and 5'-CATCCACCATGACCTCAATG-3' for hIL-12m amplification, and 5'-CTGACAATGGCAGCAATTT-3' and 5'-TCCCGCCACCAACA-3' for the common amplification of all four plasmids. As an internal control, rat b-actin was amplified using the primers 5' -TTC-AACACCCCAGCCATGT-3' and 5'-GTGGTACGACCAGAGGCATACA-3'.

In results, all male and female samples were negative for integrated plasmid. These

results suggest that GX-12 is not integrated into host chromosomal DNA, and the risk of genomic mutation due to integration into host genomic DNA is negligible.

Keyword : GX-12, genomic integration, host cellular DNA, real-time PCR