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Poster Abstract – 5.10

TRANSPLASTOMIC TOBACCO AS BIOFACTORY FOR THE PRODUCTION OF HIV-1 *GAG* ANTIGEN

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The development of an effective and safe vaccine strategy for HIV virus represents a crucial goal for both industrialized and developing countries. So far, different candidate HIV vaccine strategies have been developed. A promising approach is based on the property of the capsid protein Gag (Pr55) to auto-assemble and form Virus-Like Particles (VLPs) displaying several viral epitopes on the surface.

Recently, there is an increasing interest towards the use of plants as biofactories to produce industrial products and pharmaceuticals. In comparison with other production systems, plants show a lower cost, a higher yield, the lack of pathogens and/or toxins dangerous to human health, a higher flexibility towards changes in the product request. However, the use of transgenic nuclear plants as bio-factories generally resulted in low expression levels of antigenic proteins. To overcome these limits, we are attempting to express the capsid protein Gag (Pr55) in the plastidial genome to test the feasibility of using this technology for the production of plant-derived HIV antigen. The transformation of the plastidial genome generally shows high level of gene expression and absence of gene silencing, the possibility to coexpress several genes in an operon under the control of a single promoter, the precise integration of transgenes by homologous recombination, the containment of transgenes and gene products.

The *gag* gene from HIV-1 was cloned, with or without an N-terminal His6-tag, under the control of the strong promoter Prrn and the 5'-UTR of the T7 phage gene 10 in vectors designed for the integration of transgenes in either the inverted repeats (IR) or the large single copy region (LSC) of the plastidial genome (courtesy of Dr. P. Maliga, USA, and Dr. R. Bock, Germany). Recombinant vectors were used for biolistic transformation of *Nicotiana tabacum* (cv Petite Havana) plants.

So far, transplastomic plants were obtained with the *gag* construct without the N-terminal His6-tag. The characterization of spectinomycin and streptomycin-resistant plants by PCR and Southern analyses demonstrated the correct integration of the transgene and a high degree of homoplasmy. Northern analysis showed a high level of *gag* gene expression, whereas the analysis of protein expression with polyclonal human antibodies from blood serum of AIDS patients revealed aspecific bands both in transplastomic plants and untrasformed control. Further experiments with recombinant HIV-1-p24 antiserum are under way.