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Poster Abstract – F.09

EXPRESSION OF HPV L1 GENE IN TOBACCO CHLOROPLASTS

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Biopharmaceuticals have traditionally been produced in a variety of transgenic systems. Also plants can be used as a source of therapeutic proteins and are now considered a promising production platform. They have been shown to have several advantages over the conventional systems, such as: lower costs, easy of scalability, absence of health risks. The objective of this project is the expression in tobacco plants of a viral antigen, the L1 protein from the Human Papillomavirus (HPV), a virus which causes the cervical cancer. To date, cervical cancer is the fifth leading cause of death for women worldwide. L1 gene codes for the major capsid protein which forms Virus Like Particles (VLP). VLPs mimic the infection with virions and induce virus-neutralizing antibodies. Thus, VLPs are the most attractive candidate for developing a prophylactic vaccine against HPV infections. To overcome the limit of a low yield of recombinant protein, we used plastid transformation, which allows to deliver the transforming DNA in the plastid genome, rather than the nucleus. Compared to conventional transgenic technologies, plastid engineering generally shows higher protein expression levels, no gene silencing, transgene containment due to the absence of plastids in the pollen.

Several vectors containing the wild-type L1 sequence were constructed, in which L1 is fused to either the GST or the His tag and regulated by strong plastid promoters and different 5'UTRs. In order to test the effect of codon usage and nucleotide sequence on transgene expression, the gene sequence was also changed according to the plastid codon usage and four new vectors carrying the plastid modified sequence were assembled. Spectinomycin resistant transplastomic plants were obtained following biolistic DNA delivery. Correct gene insertion and homoplasmy level were tested by PCR and Southern blot analyses.

RNA gel blot analyses were carried out to verify the presence of L1 transcripts in tobacco plastids. The presence of both monocistronic and dicistronic messages was shown. Western blot analysis carried out on *E. coli* total cellular proteins using an anti-His antibody showed that L1 protein largely accumulates in the insoluble fraction of *E. coli*. By contrast, no protein was detected in plant extracts by western blot using either anti-tag or anti-L1 antibodies. Pulse-chase labeling experiments demonstrated that the protein slightly accumulates in plastids, but it is highly unstable, since the signal decreases after 4 hour chase, almost disappearing after 24 hours.

Supported by recent data found in literature, new vectors with a human-optimized version of the gene are under construction.