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PLANTS AND PLANT ROOT CULTURES AS PLATFORMS FOR PRODUCING THERAPEUTIC VACCINES

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Two prophylactic vaccines (Gardasil-Merck, Cervarix-GSK) are available against the 'high risk' human papillomaviruses (mainly HPV16 and 18), the primary etiologic agents of cervical cancer. A therapeutic vaccine against cervical cancer would help also the cure of other HPV-associated pathologies (i.e. tonsillar carcinomas, penis cancers and head and neck tumors) and would involve already infected women (about 400 million worldwide). Recently, Roche and Transgene entered partnership to develop a Phase III study with the aim to commercialize a therapeuthic vaccine against HPV-mediated diseases (TG4001, based on HPV16 E6 and E7 tumor-specific antigens).

Plant bio-farming and recombinant DNA technologies hold promise being low-cost production technologies and representing platforms to improve the poor immunogenicity of tumor antigens. This issue is of particular relevance for HPV associated pathologies because cervical cancer represents the main cause of cancer-related death in women in developing countries where the economies cannot sustain high expenses for health.

We recently utilized *Nicotiana benthamiana,* a non-food plant, as a platform for the production of a therapeutic vaccine consisting of a fusion protein made by engineering HPV16 E7 coding sequence as a fusion to β-1,3-1,4-glucanase (LicKM) of *Clostridium thermocellum*. The vaccine was produced by using a transient expression system. Target antigen was purified and evaluated in a mouse model for its potential as a therapeutic vaccine. The experimentations were carried out on a large number of animals (i.e. 25-50 per treatment) in order to avoid possible variations linked to the variability of the biological response. The vaccine induced E7-specific IgG and cytotoxic T-cell responses inhibiting tumor development following challenge with an E7-expressing tumor cell line. The data were collected from animals treated with different administration schedules demonstrating the strong effectiveness of plant-produced vaccines.

In parallel, the presence of LicKM in the E7 hybrid proteins is currently being exploited to facilitate the analysis and the selection of high-rate expressing clonal roots obtained from stable transformation of leaf explants from *Petunia hybrida* and of *Nicotiana benthamiana* with recombinant *Agrobacterium rhizogenes*. Organ cultures have been obtained in order to produce

extracts, lyophilized material or purified fusion proteins to be tested in a pre-clinical vaccination model.

In conclusion, our formulations, being based on purified antigens that might also be produced by *in vitro* cultures, could shorten the time needed for the application of plant-derived vaccines also in humans.