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Poster Abstract – F.08

EXPRESSION IN PLANTS OF PROTEINS FROM HPV8, A CUTANEOUS HUMAN PAPILLOMAVIRUS

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Non melanoma skin cancer (NMSC), including basal cell (BCC, 80% of NMSC) and squamous cell carcinoma (SCC, 20%) is the most frequent cancer in Caucasians. Its incidence has dramatically increased in the last years due to sun exposure and immunosuppression: i.e. transplant recipient have a 100-fold and a 10-fold risk increase of developing SCC and BCC, respectively. Sensitive diagnostic techniques have detected a few Human Papillomavirus (HPV) species in lesions of patients affected by these tumours, particularly belonging to the Beta-papillomavirus Genus. This Genus includes viruses with a cutaneous tropism, while high risk HPVs , responsible of almost all cervical cancers in woman, have a mucosal tropism. A few Beta HPVs, such as HPV8 and HPV5, are found associated with skin cancer lesions and are thus considered etiologically related to these pathologies.

For preventing mucosal HPV infection, prophylactic vaccines based on the viral L1 major capsid protein, assembled into empty virion like particles are already available. Preventive vaccines targeting non structural viral proteins, such as the oncogenic proteins E6 or E7 are also under study. Instead, for cutaneous HPVs, no vaccine strategies have yet been described.

Plants have been proposed as alternative platforms for producing foreign antigens for vaccine production and immunisation purposes. Antigens expressed in plants can be administered in different ways, including direct oral ingestion of plant tissue (edible vaccine).

We have recently undertaken a project aimed at obtaining vaccines against cutaneous HPVs, based on the expression in plants of the major L1 capsid and of the E7 protein of HPV 8. To evaluate protein expression in plants tissue, polyclonal antibodies were raised in rabbits against purified GST-L1 and GST–E7 fusion proteins, produced in *E. coli*.

For the expression in plants, 35S-based cassettes for (1) the full length L1 protein, (2) an L1 protein deprived of a putative nuclear localisation signal, (3) the E7 protein and (4) an L1-E7 fusion construct were thus generated and used for both transient and stable expression experiments. For transient expression, infiltration of *N. benthamiana* leaves with *A. tumefaciens* carrying the different constructs (agroinfiltration) was carried out. Transgene expression was followed both at the RNA and protein level. Simultaneous infiltration of agrobacteria carrying genes for silencing suppressors, such as p19 from *Cymbidium ringspot virus*, and/or HC-Pro from *Potato virus Y* enhanced transgene expression of all constructs. Data concerning the molecular characterisation of *N. benthamiana* lines transgenic for the L1 and E7 antigens and their derivatives will be presented.