Global approach to improve management and therapy of HPV-related cancer

57

Francesca Paolini[1], Anna Maria Mileo[1], Luciano Mariani[2], Aldo Venuti[1]
[1]UOSD Tumor Immunology and Immunotherapy; [2]UOC Gynecology,
IRCCS Regina Elena National Cancer Institute, Rome, Italy

American Cancer Society has launched a public health campaign with one very ambitious goal: to eliminate vaccine-preventable HPV cancers, however many questions remain unresolved in HPV field. It is estimated that HPV-related cancers account for 5% of all human cancers. Current HPV vaccines are extremely effective at preventing infection and neoplastic disease; however, they are prophylactic and do not clear established infections. Thus, the need to improve therapeutic options for the billions of already infected patients worldwide. Cervical screening is a pivotal tool for management of cervical cancer prevention and early treatment. HPV testing is replacing pap test as screening method, but we still have to ameliorate our technologies to improve HPV test for screening and to develop new triage methods

We herein propose a global approach with **two main objectives:**

a) New immunotherapies (DNA therapeutic vaccines)

Previous studies (Massa et al, Hum Gene Ther 2008; Massa et al, Hum Vaccin 2011; Paolini et al, Hum Vaccin Immunother 2013) on plant produced HPV antigens gave evidence of their immunogenicity and efficacy in animal models and led also to patent release. Exploiting the signal sequence of the Polygalacturonase-inhibiting protein (PGIPss) from Phaseolus vulgaris, we targeted the HPV16 E7 protein to the plant secretory compartment (Franconi et al, Int J Immunopathol Pharmacol 2006) (Fig.1). PGIPss, fused to N-terminal portion of a HPV16 antigen, was able to modify the antigen compartmentalization/processing in HEK-293 cells, promoting its secretion and demonstrating for the first time that a plant ss may work in mammalian cells. (Massa et al. Hum Vaccin Immunother 2017) (Fig. 2).

Fig.1

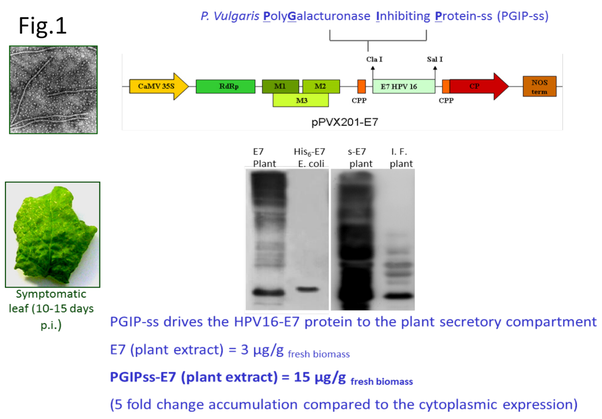
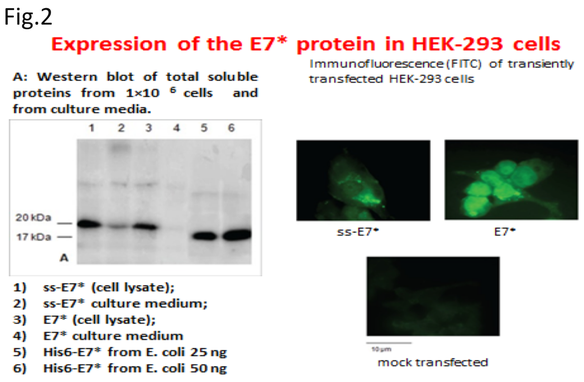
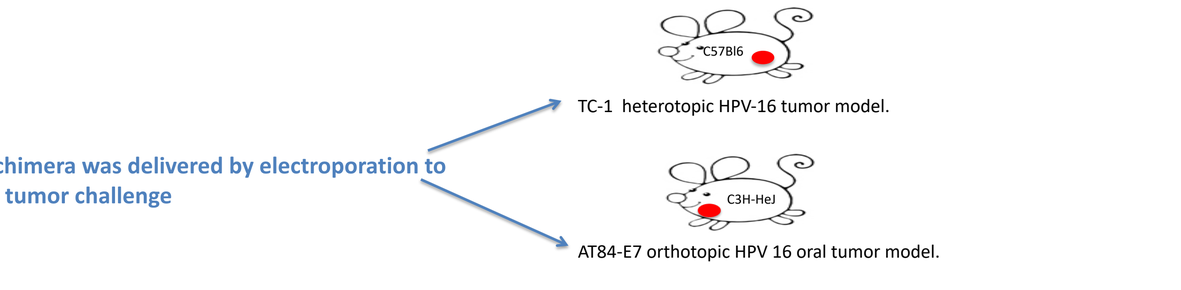


Fig.2



A chimeric construct consisting of L2 (first 200 aa.)-E7 of HPV16 was fused to PGIPss and cloned in pVax vector to develop a preventive/therapeutic vaccine (Fig.3)

Fig.3



This chimeric PGIPss-L2-E7 construct induced strong and long lasting immunological responses. Serum (ELISA Fig. 4A and B) and cell-mediated (ELISPOT of IFNγ producing splenocytes; Fig. 5) responses were detected after treatment with the chimera. In addition, mouse sera showed long-lasting maintenance of the acquired immunity (WB; Fig. 6).

Fig.4

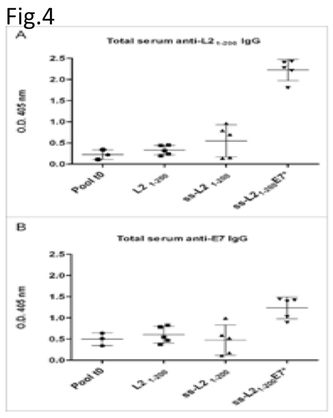


Fig.5

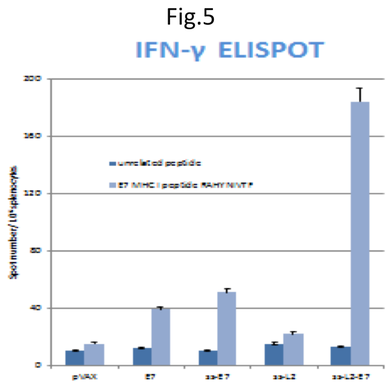
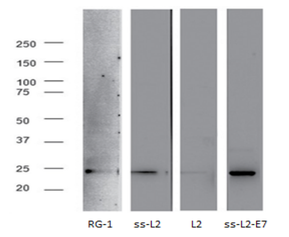
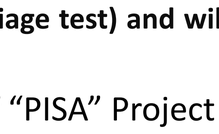


Fig.6



Chimeric PGIPss-L2-E7 construct induced a dramatic reduction of tumor growth

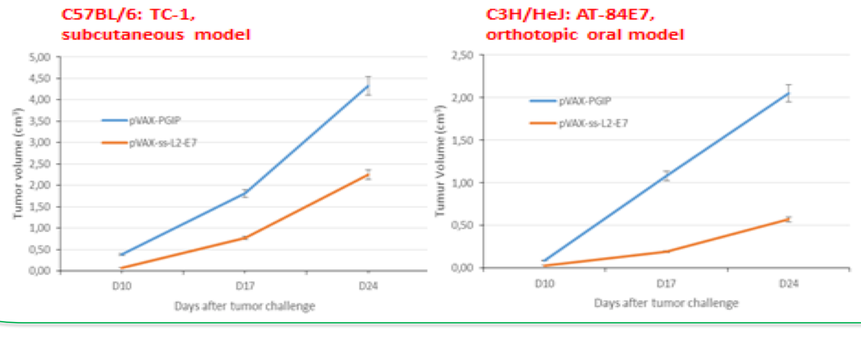
The chimera was delivered by electroporation to after tumor challenge



TC-1 heterotopic HPV-16 tumor model.

AT84-E7 orthotopic HPV 16 oral tumor model.

Anticancer responses in pre-clinical mouse models upon treatment with the chimera

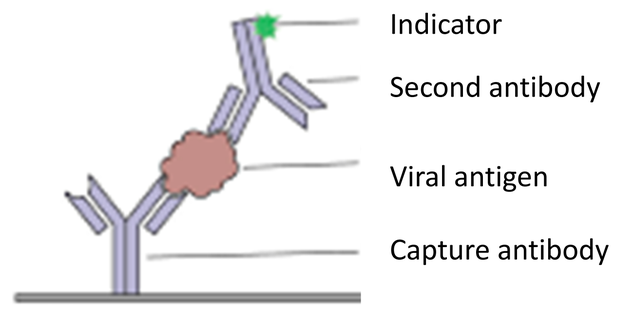


b) New diagnostic tools

Detection of E6/E7 oncogene proteins in clinical samples and of serum antibodies against same oncogenes. We are developing, in collaboration with Nanofaber Company, two device prototypes (i) for detection of viral oncoproteins and (ii) for determination of circulating antibodies against the same oncoproteins.

(i) For detection of oncoproteins in biological samples, two strategies are ongoing:

1) a conventional strategy based on the immobilization of antibodies against E6 and/or E7 oncoproteins on a polymeric surface (Nanofaber) obtained by electrospinning to bind the viral antigen and a specific enzyme-conjugated secondary antibody for colorimetric or fluorescent detection/quantification.



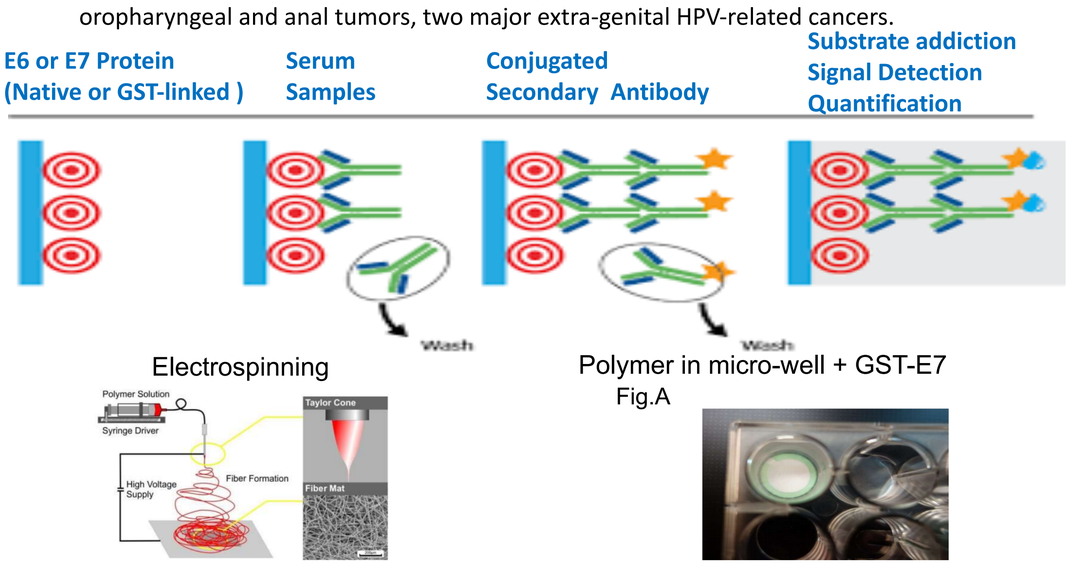
Property Nanofaber solid support

2) an innovative strategy that exploits the capacity of only high risk HPV oncoproteins to bind some cell substrates, different PDZ proteins for E6 or retinoblastoma protein (pRb) for E7. These binding proteins are immobilized on the chip and, when viral oncoprotein is present, a "sandwich" is formed that allows the detection.

Property Nanofaber solid support

(ii) For determination of circulating antibodies, a device based on the same technology is ongoing

The oncoproteins E6 and/or E7 of HPV16 are immobilized on same polymer surface. The choice of this HPV type is motivated by the association of this HPV with almost all oropharyngeal and anal tumors, two major extra-genital HPV-related cancers.



Preliminary results.
IgG antibody levels in a human serum already known for its HPV16 E7 positivity (Di Lonardo et al. J Med Virol 1998)

Sample ID*	Antibody level absorbance (405 nm) Mean (SEM)
(1998) HPV16/E7	0.265 (0.0005)

*Samples (dilution 1:50) were considered positive with absorbance values higher than 0.1. GST-E7 was utilized as antigen spotted on polymer in micro-well (Fig. A)

Conclusions

Our chimeric DNA vaccine (Patent pending) is a promising tool for preventive-therapeutic vaccination, particularly useful in patients already infected by HPV and in low-income countries.

The new diagnostic tools based on the detection of oncoproteins (directly or through the measurement of specific antibodies) will improve the diagnostic performance of HPV tests increasing their predictive positive significance (triage test) and will open perspectives for rapid analysis of self-collected samples (by immersing a stick format of our device directly in the self-collected sample).

These studies are part of “PISA” Project granted by “Progetti Gruppi di Ricerca - finanziamento regione, LAZIO INNOVA spa”.