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► **To cite this version:**

Gabrielle Romain, Ellen Van Gulck, Gérard Zurawski, Jacques Banchereau, Guido Vanham, et al.. P18-08. Characterization of CD34+ derived dendritic cells generated in vitro and transfected with HIV gene as potential therapeutic vaccine in macaque. AIDS Vaccine 2009, Oct 2009, Paris, France. BioMed Central, 6 (Suppl 3), pp.P317, 2009, Retrovirology. <10.1186/1742-4690-6-S3-P317>. <inserm-00668483>

**HAL Id: inserm-00668483**

**<http://www.hal.inserm.fr/inserm-00668483>**

Submitted on 9 Feb 2012

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## **PI8-08. Characterization of CD34+ derived dendritic cells generated *in vitro* and transfected with HIV gene as potential therapeutic vaccine in macaque**

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from AIDS Vaccine 2009  
Paris, France. 19–22 October 2009

Published: 22 October 2009

Retrovirology 2009, **6**(Suppl 3):P317 doi:10.1186/1742-4690-6-S3-P317

This abstract is available from: <http://www.retrovirology.com/content/6/S3/P317>

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### **Background**

Antiretroviral therapies against HIV infection reduce the plasma viral load but can not eradicate the virus. Dendritic cells (DC) transfected with messenger ribonucleic acids (mRNA) encoding endogenous viral proteins are expected to enhance the HIV specific immune response and are considered as potent therapeutic vaccines. In this context we studied the feasibility and efficiency of mRNA loaded CD34+ derived DC as therapeutic vaccine in SIV infected macaques as a model of HIV infection and AIDS.

### **Methods**

DC were derived from macaque medullar CD34+ cells by *in vitro* proliferation for 7 days with early acting cytokines, IL-3 and IL-6 and by differentiation for 7 days with GM-CSF and IL-4. To mature the cells, a cocktail consisting of pro-inflammatory cytokines was added for 24 hours. Mature DC were transfected by electroporation with human codon optimized HxB-2 Gag mRNA. Two hours after electroporation, cells were frozen until use for vaccination. In a preliminary study, uninfected animals received 4 injections 4 weeks apart of  $15 \times 10^6$  autologous transfected DC, administered both intradermally and subcutaneously. Immunomonitoring was focused on the detection of Gag-specific antibodies and IFN- $\gamma$  and IL-2 secreting cells in peripheral blood.

### **Results**

This process yielded 10 to 60 fold more DC than the input number of CD34+ cells. The electroporated DC express CD83, CCR7; high level of HLA-DR, CD40, CD86, CD1a, CD1d, ASGPR and CLEC-6; and lower level of DC-SIGN, Dectin-1, Lox-1 and DCIR. After thawing, 90% cells were alive and 70% expressed Gag. Two weeks after the first vaccination, peripheral blood cells evidenced strong production of IFN- $\gamma$  and IL-2. This indicated that it is possible to induce polyfunctional T-cells.

### **Conclusion**

This opens perspectives for the use of CD34+ derived DC electroporated with mRNA encoding HIV-gag as therapeutic vaccine in macaque model.