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**Training on multiparametric ELISPOT based measurements for the evaluation of a novel VEGF based cancer immunotherapy.**

### **Abstract**

Active immunotherapy for cancer is an avenue under expansion holding large promises for the development of breakthrough treatments for this group of diseases. Some active therapies have been designed with the primary objective of inducing antibodies leading to the depletion of the circulating targets molecules. In the last decade at CIGB in Havana, the Cancer immunotherapy group has developed a novel VEGF targeted strategy, HEBERSaVax. This therapy combines the induction of anti-angiogenic antibodies able to deplete circulating VEGF and the induction of a VEGF specific T cell response. This dual effect has a combination of a) circulating factor neutralization and depletion and b) cytotoxic effects on the stromal and tumour cells aberrantly producing large amounts of the VEGF that in turn fuel tumour angiogenesis, immunosuppression, and metastasis.

In our strategy to characterize the immune response to HEBERSaVax in humans, several attempts to improve the quality of the evaluation of the frequency of IFN gamma and GRZ-B secreting T cell clones within PBMC from immunized patient samples have been made. So far, our analyses are limited to manual counting and the use of ELISTAT software coupled to ELISPOT reader. These strategies offer neither the automatization nor the independence from human bias needed for this type of study.

The project herein proposed aim at acquiring expertise on the evaluation/analyses of ELISPOT/FLUOROSPOT plates using CTL immunospot reader and software. To this end, the project is divided into two short-time learning objectives followed by the another two related to their practical application in the analysis of the kinetics of IFN gamma and GRZB of serial samples collected from approximately 100 patients.