Innovative strategy against glioblastoma: Therapy based on *Neospora caninum* armed with therapeutic antibodies

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Glioblastoma

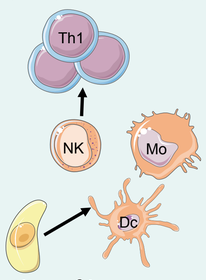
Glioblastoma (GBM) is the **most common and aggressive malignant brain tumor** in adult and represents **14,5% of all primary brain and other central nervous system tumors**. Conventional treatments for this cancer are Surgery, Radiotherapy and Chemotherapy. The treatment of this tumor is systematically followed by a **relapse of the disease** and the prognosis of patients with glioblastoma is poor with a **median life of 15 months** and a **five-year survival rate of 5%**[1]**.**

In the case of melanoma or lung cancers, which present a very **aggressive character** and a **strong angiogenesis**, the use of therapeutic antibodies has greatly increased the survival of patients and immunotherapy has fundamentally changed the treatment of these cancers. In the case of GBM, **immunotherapy was disappointing** with limited therapeutic effect and inability to increase the average survival of patients. This resistance to immunotherapies involve several mechanisms: **immunosuppressive tumor microenvironment**, **intrinsic resistance** of tumor cells to treatment or environmental parameters such as hypoxia. **Therefore, development of new treatments and strategies to fight this cancer is still needed**[2].

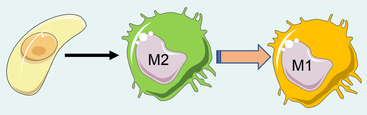
N. caninum used as anti-tumor agent

The BioMAP team proposed a new innovative immunotherapeutic treatment for cancer with the use of this intracellular unicellular eukaryotic organism[3]. The anti-tumor activity was studied in a murine thymic lymphoma model and involves 3 mechanisms of action:

-Direct oncolytic activity



-Induction of immune response

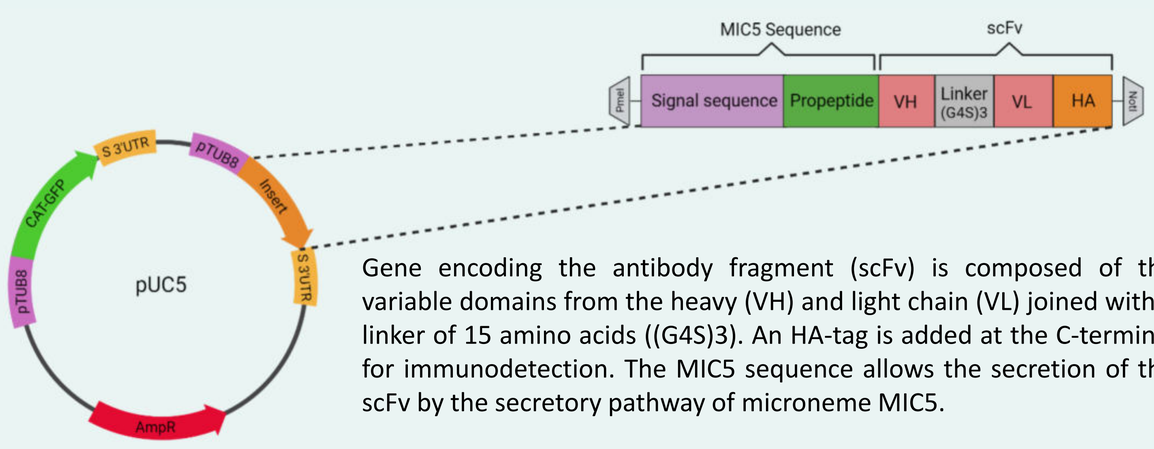


-Reprogrammation of the tumor microenvironnement

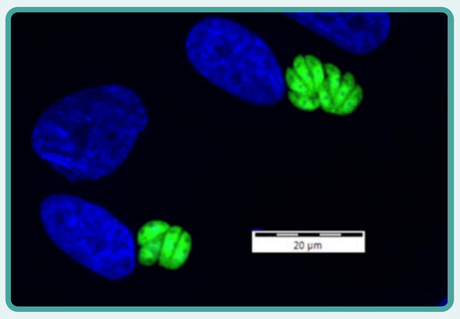
These encouraging results led us to use this protozoan to vectorize therapeutic molecules more particularly therapeutic antibodies.

Production of recombinant strains

The vector pUC5 contained 2 expression cassettes under the control of the *T. gondii* tubulin promoter (pTUB). The first allows the production of the chloramphenicol acetyl transferase (CAT) fused with the Green Fluorescence Protein (GFP) for selection of recombinant tachyzoites. The second cassette is used for the expression of the protein of interest.



Gene encoding the antibody fragment (scFv) is composed of the variable domains from the heavy (VH) and light chain (VL) joined with a linker of 15 amino acids ((G4S)3). An HA-tag is added at the C-terminal for immunodetection. The MIC5 sequence allows the secretion of the scFv by the secretory pathway of microneme MIC5.



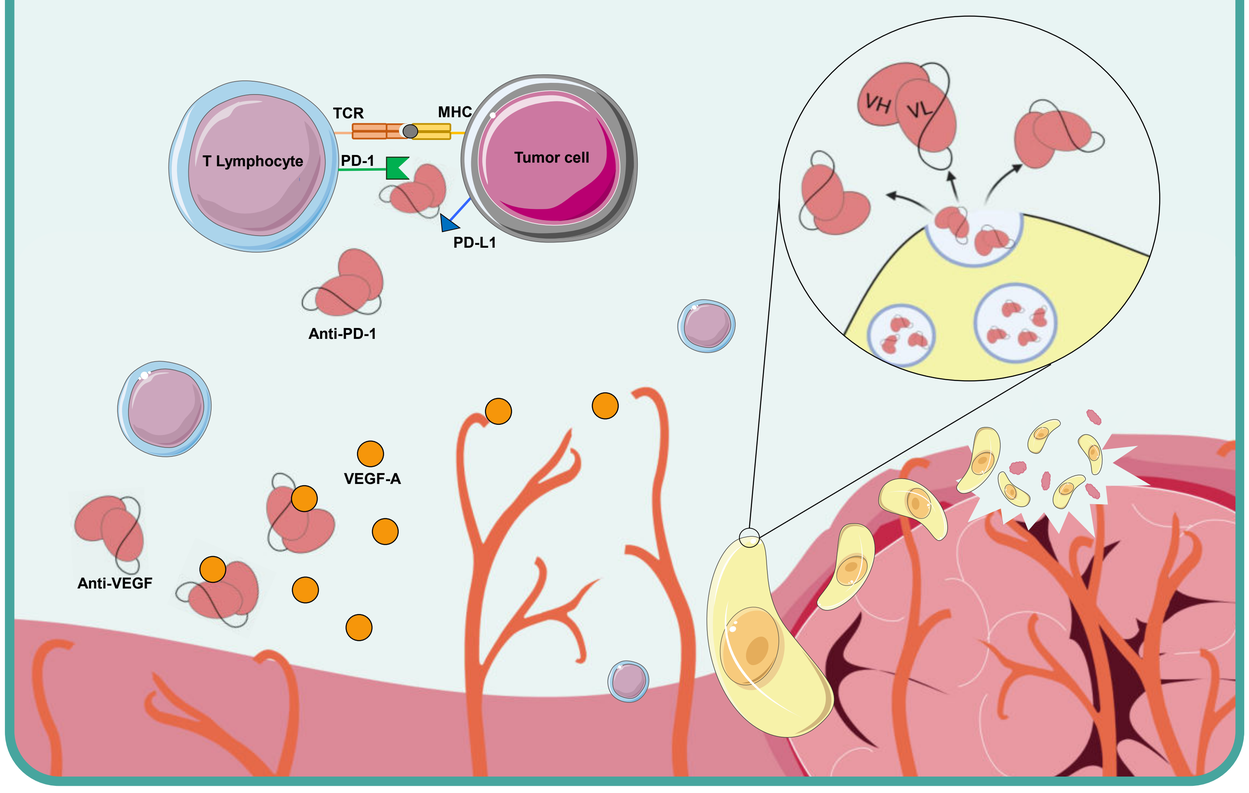
After transfection and few passages under selection of Chloramphenicol, *Neospora caninum* tachyzoites expressing GFP are observed.

➔Obtention of stable recombinant strains

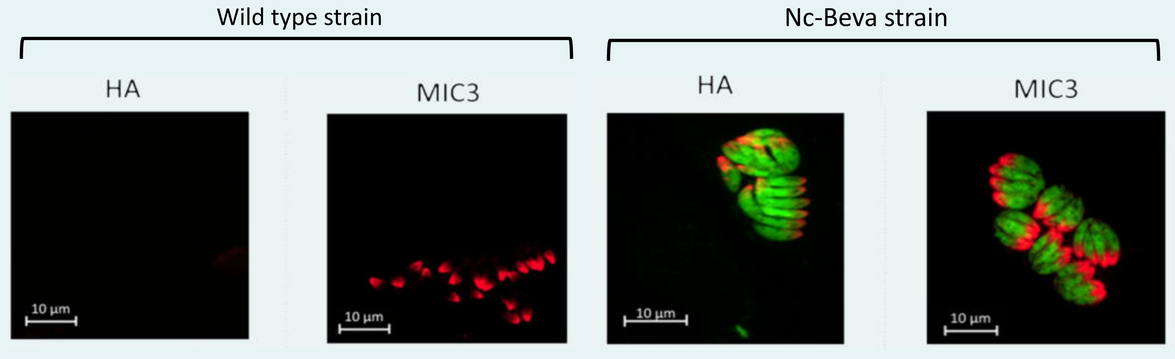
Objectives of the project

The goal of the project is to increase the antitumor properties of *Neospora caninum* by developing recombinant strains secreting an antibody: anti-VEGF (Bevacizumab) **or** anti-PD1 (Pembrolizumab).

Bevacizumab and Pembrolizumab directly bind and neutralize respectively VEGF-A and PD-1.
The first prevents the activation of VEGF receptor and controls angiogenesis with a normalization of blood vessels.
The anti-PD-1 neutralizes its target and prevents the interaction of PD1 and PD-L1 which is involved in the negative regulation of T lymphocyte activity.



Proximity with microneme protein



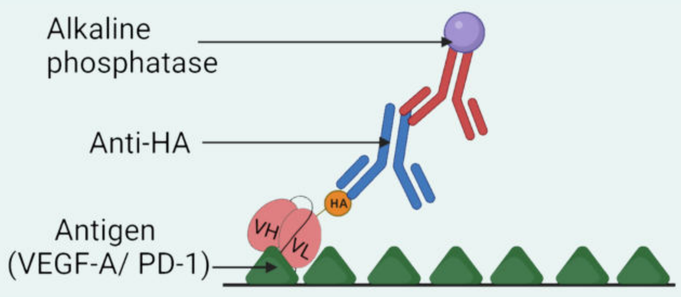
Immunostaining of recombinant *Neospora caninum* using anti-HA or anti-microneme protein MIC3 shows an apical staining for both MIC3 and the tagged scFv anti-VEGF.

➔Suggesting a secretion of the antibody fragment by the microneme pathway

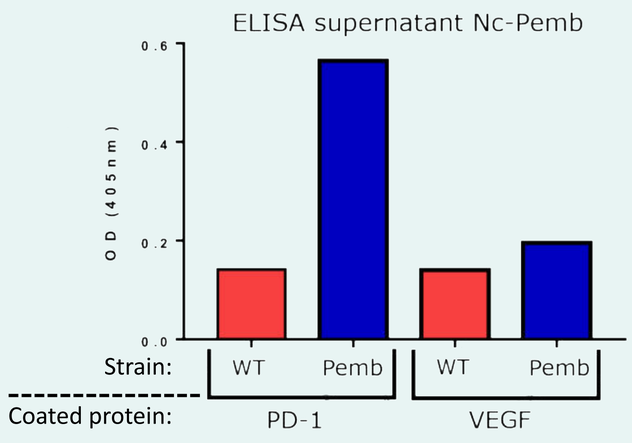
Secretion of antibodies

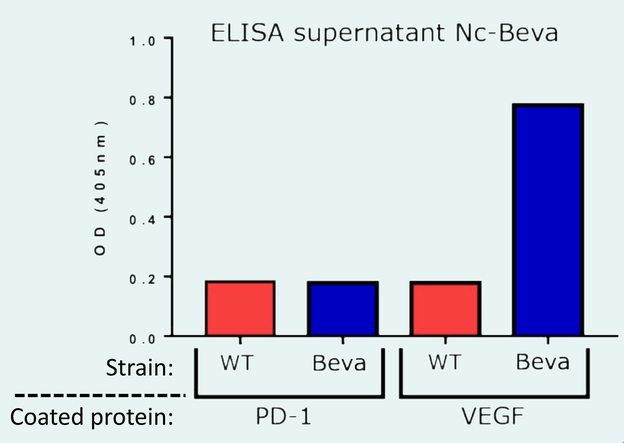
The secretion of anti-VEGF (NC-Beva) or anti-PD-1(Nc-Pemb) expressed by the protozoan has been evaluated in the culture supernatant of recombinant strains.

ELISA assays with coated recombinant VEGF or PD-1



➔ These scFvs secreted by *Neospora caninum* are able to specifically bind their target





Conclusion and perspectives

These results show the ability to *Nesopora caninum* to produce and to secrete functional antibody fragments like anti-VEGF-A and anti-PD-1. Next objectives are I) to determine the quantity of antibody expressed by the protozoan, II) to validate *in-vitro* the neutralizing activity of these both antibodies and III) to study i*n-vivo* the anti-tumor activity of these different *Neospora caninum strains*. This project is an opportunity to improve the treatment of Glioblastoma with the possibility to develop many therapies with other antibodies and in different format like scFv-Fc.

Bibliography:

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