Targeting tumor associated macrophages (TAM) with vectorized magnetic nanoparticles for anticancer therapies

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Tumor associated macrophages (TAMs) are capable to confer anti-apoptotic, proliferative and enhanced migratory properties to cancer cells, as well as protecting them against immune attacks and therapies. In lung cancer, they represent between 30 to 50% of the tumor mass and they are associated with a bad prognosis of the disease [1,2]. Targeting these pro-tumoral TAMs is a major challenge in anticancer therapies. Different strategies exist, but they are not specific, potentially leading to adverse effects. Our team produced and patented a monoclonal antibody, 6-25, capable to specifically recognize pro-tumoral TAMs displaying an M2 phenotype. We showed that the 6-25 mAb was internalized in these TAMs without inducing any toxicity. The goal of the project is to produce a tool that specifically targets and kills pro-tumoral TAMs.

In cancer treatment, magnetic hyperthermia or magnetomechanical ablation represent an emerging approach with promising therapeutic potential. The first one induce cell death for cell containing magnetic nanoparticles by increasing the temperature through a high frequency alternating magnetic while the second uses mechanical forces under low frequency rotating magnetic field (MF) [3]. We therefore developed a biocompatible and non-toxic magnetic nanoparticle functionalized with the 6-25 mAb (MNP-6-25) as a specific tool to target pro-tumoral TAMs in the tumor.

To test the feasibility of this method, we performed a robust 3D model of co-cultures with the lung cancer cell line (A549) with M2 macrophages (M2M), or M1 macrophages (M1M) as a negative control, these cells doesn't express the target of 6-25. We have studied the basal cytotoxicity, kinetics and specifity of binding of the MNP-6-25 for M2M in these models using flow cytometry and microscopy.

First, we showed that MNP-6-25 are not toxic to neither M2M nor M1M in a concentration up to 64 µg Fe2O3/mL after 72h of incubation, and bind specifically M2M but not M1M, with a maximum at 48h of incubation at 8 µg/mL (Fig. 1). We developed 3D models with cancer cell line and M2 macrophages derived from monocytes. The M2 phenotype of macrophages is stable in our model over seven days with an increase of the CD204 M2 marker in the 3D model and M2M promote tumor cell proliferation (Fig. 2). Replacing M1M in the 3D model promotes A549 proliferation. This is consistent with the depolarization of M1M towards an M2 phenotype.
Flow cytometry results and microscopy show the specific binding of MNP-6-25 to M2M but not to M1M in 2D and 3D models, also compare to control nanoparticles: non-vectorized nanoparticles or vectorized with the isotipic control of the 6-25 antibody (Fig. 3).

To conclude, we showed that MNP-6-25 specifically target M2M inside 2D/3D heterotypic models. Future research will focus on the induction of TAM death in these *in vitro* models, as well as on the targeting and death of TAMs in an *in vivo* model, which has already been set up.

References

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Figures

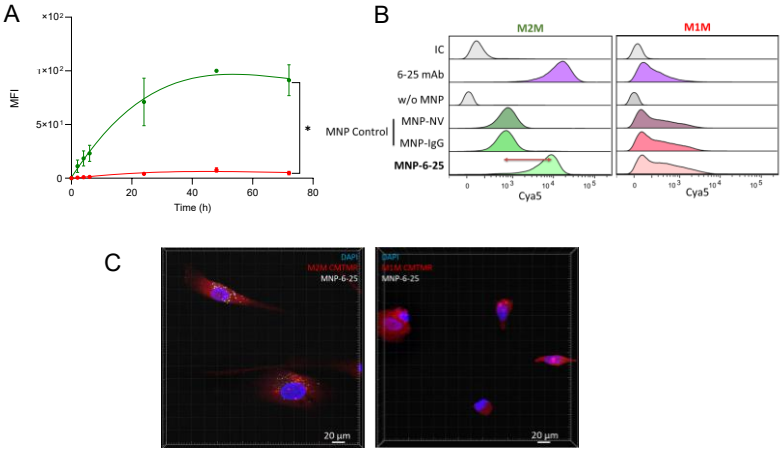

Figure 1: Specific targeting of M2M in monoculture by MNP-6-25.
A - Kinetics of MNP-6-25 binding to M1M (red line) and to M2M (green line) incubated with 8 µg γ-Fe2O3/ml of fluorescent MNP-6-25 for 2, 4, 6, 24, 48 or 72h at 37°C. B - M1M and M2M incubated with

MNP vectorized (with IgG for isotypic control or 6-25 mAb) or not (NV - non-vectorized) at 16 µg/ml, then analyzed by flow cytometry. C - M1M and M2M (CMTMR staining, red) incubated with MNP-6-25 (white), then fixed and stained with DAPI (blue) before analysis by confocal microscopy.

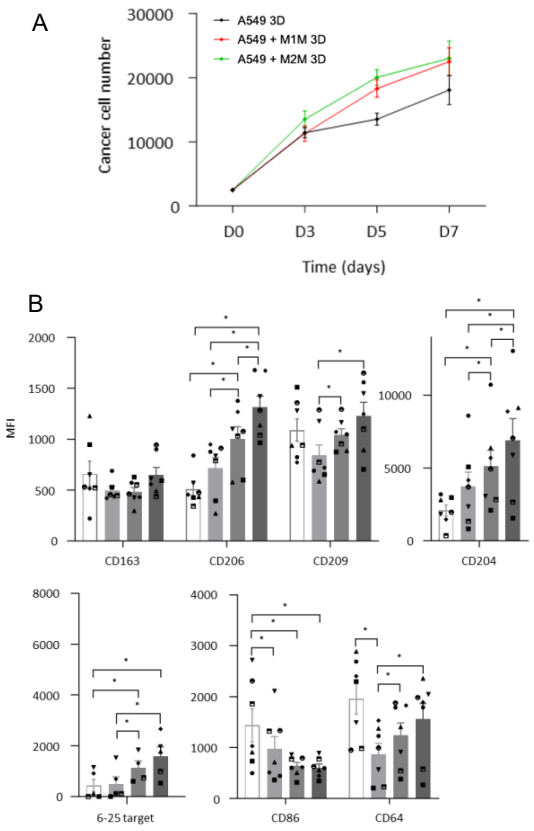

Figure 2: M1M in 3D models with cancer cells are depolarized toward an M2 phenotype and favor cancer cells proliferation.
A – Proliferation of A549 in 3D co-cultures with M1M or M2M for 7 days. B – Expression of CD163, CD206, CD209, CD204, 6-25 target, CD64 and CD86 measured by flow cytometry on M1M in co-culture 3D.

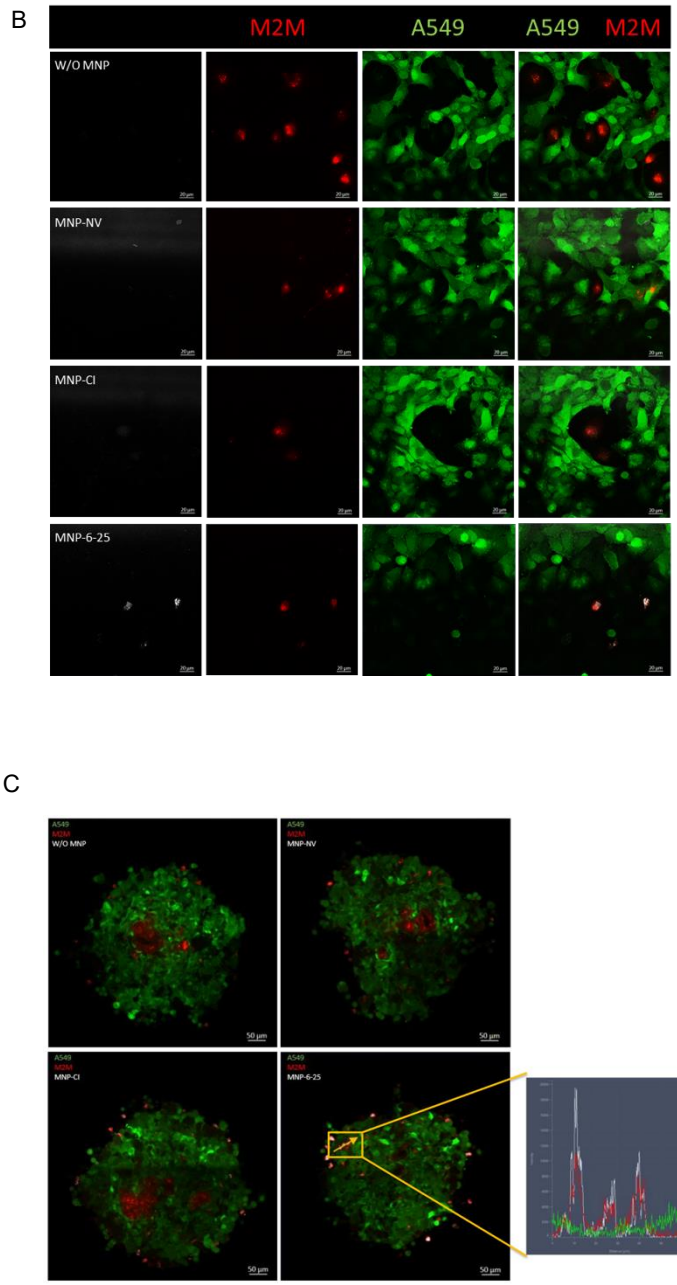

Figure 3 : High specificity of binding of MNP-6-25 to M2M in 2D and 3D co-cultures. A – 2D or 3D co-culture of A549 with M2M were incubated with various forms of MNPs at 16 µg/mL (NV – non-vectorized and IgG – isotypic control) and subsequently analyzed by flow cytometry. B/C – Confocal images of 2D (B) and 3D (C) co-culture models (A549 GFP – M2M CMTMR) incubated 72h with different magnetic nanoparticles : MNP-NV, MNP-CI, MNP-6-25 at 16 µg/mL (colocalization profile: arrow in the square).