Use of monoclonal antibodies for therapeutic purposes necessitates a careful choice of subclasses and isotypes to fulfil the desired functional effect *in vivo*.

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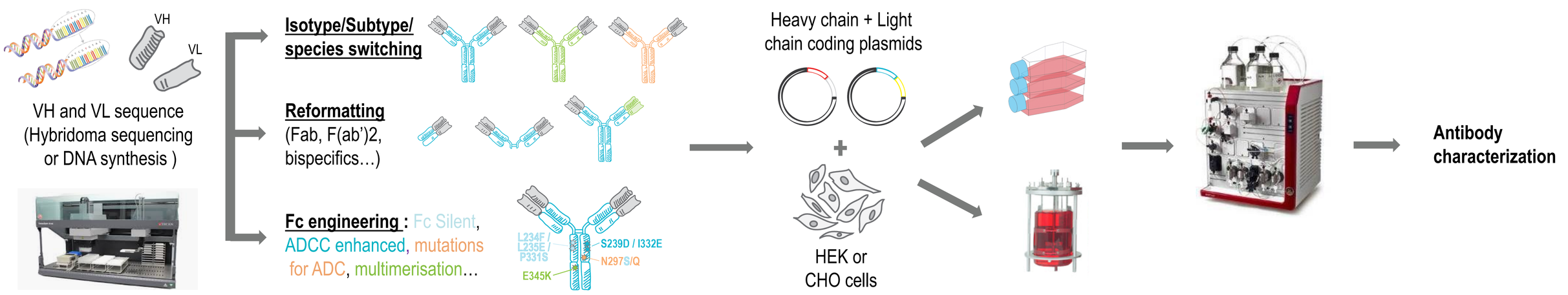
Abstract

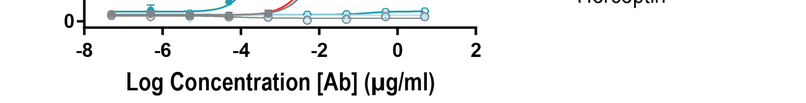
During the last decade, monoclonal antibodies have emerged as new effective therapeutic agents. They are becoming one of the largest class of newly approved drugs for the treatment of many diseases. In this context of continuously increasing demand , MI-mAbs, an innovative immunotechnological center, was designed to help academics and industrials to accelerate the development of such new therapeutic antibodies (Abs).
In immuno-oncology, depending on the therapeutic context, monoclonal antibodies (mAbs) have to be immunomodulatory, agonist, antagonist, activatory or blocking agents. Therefore, modulating their immune effectors functions is of crucial importance either by choice of the appropriate isotype or by some carefully selected Fc engineering.
In a direct tumour killing purpose, depletion of target cells is usually performed using human IgG1 prone to complement and ADCC (Antibody Dependant Cell Cytotoxicity) effects; this subclass can be further mutated to enhance these functions. Conversely, in a growing number of applications (immune cells receptor targeting, Antibody Drug Conjugate, bispecifics…), these Fc mediated immune function may be undesirable: mutated Fc silent IgG1 are, in such cases, variants of choice compared to natural isotypes.
At MI-mAbs, considering this growing interest for Fc modulating (fine-tuning) functions, we produce a variety of subclasses and isotypes with different properties. We will illustrate the importance of such different formats with two different examples, comparing *in vitro* and *in vivo* efficacy of 3 "reformatted" antibodies: firstly, the *in vitro* ADCC potential of an anti-CD20 and an anti-Her2 mAb under 3 formats and secondly, the follow-up of a syngeneic mouse model treated with an anti-PD1 mAb under 2 formats.

Recombinant Antibody formats



In vitro validation of antibody format potency

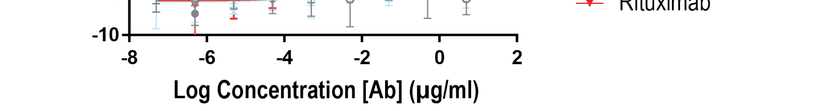
To illustrate the use of human IgG1 and some of its mutated formats for therapeutic purposes, we compared the *in vitro* efficacy of two commercial antibodies, the anti-human HER2 humanized antibody trastuzumab (Herceptin®) and the anti-CD20 chimeric antibody rituximab (Rituxan®), reformatted under 3 different isotypes: wild type IgG1 (G1), Fc-silent (S1: mutation N297S) and ADCC-enhanced (X1:mutations S239D/I332E) formats.



	Hut_G1_HER_Hercep	Hut_S1_HER_Hercep	Hut_X1_HER_Hercep	Herceptin®
Mean EC50 **(pM)	67.17	-	4.57	70.9

Figure 1: ***In vitro* efficacy comparison of an anti-Her2 antibody under 3 different formats**

ADCC activity of NK cells is measured in a **Calcein–release assay**. Specific lysis % of SKOV-3 cells (=target cells expressing hHER-2) by purified NK cells (=effector cells) in presence of the antibody of interest (after 4h incubation at +37°C) is followed. Antibody concentration range: 5 x10^{-8} to 5 µg/mL. EC50 value is calculated using Prism software (GraphPad).
**, Specific lysis % of Target cells = 100 × (E − S) / (M − S) with E, experimental lysis, S, spontaneous lysis, M, maximum lysis.*
***, Mean EC50 value was calculated from 3 independent donors.*
Effector cells :Target cells ratio = 10:1.



	CHt_G1_C20_Ritux	CHt_S1_C20_Ritux	CHt_X1_C20_Ritux	Rituxan®
Mean EC50 **(pM)	58,7	-	2,68	21.2

Figure 2: ***In vitro* efficacy comparison of an anti-CD20 antibody under 3 different formats.**

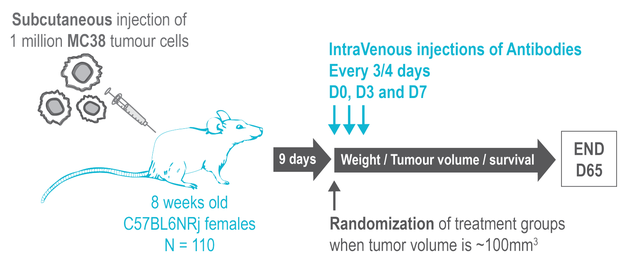
ADCC activity of purified NK is measured using **a bioluminescent reporter bioassay**. Specific Lysis % of reporter target cells (here, Raji cells that constitutively express CD20 and engineered to express luciferase) in presence of the antibody of interest (after overnight incubation at +37°C) is followed. Antibody concentration range: 5 x10^{-8} to 5 µg/mL. EC50 value is calculated using Prism software (GraphPad).
**, Specific lysis % of Target cells = 100 × (E − S) / (M − S) with E, experimental lysis, S, spontaneous lysis, M, maximum lysis .*
***, Mean EC50 value was calculated from 3 independent donors.*
Effector cells :Target cells ratio = 10:1.

- **Wild type IgG1 «reformatted» recombinant antibodies showed activities comparable to commercial antibodies (Herceptin® & Rituxan®) in ADCC assays.**
- **As expected, with Fc-silent formats (HUt_S1_HER_Hercep & CHt_S1_C20_ritux), no specific lysis was observed. The abrogation of the glycans at the main N-glycosylation site of the IgG1 Fc fragment causes a loss of cytotoxic activity of the antibody.**
- **On the contrary, ADCC-enhanced formats (HUt_X1_HER_Hercep & CHt_X1_C20_ritux) are 10 times more potent than their IgG1 counterparts.**

Indeed, S239D/I332E mutations are known to enhance FcγRIIIa affinity thus increasing ADCC potential of so engineered antibodies.

In vivo study of "reformatted"anti-PD1 surrogate

In preclinical studies, the widely used anti-mouse PD1 surrogate (clone RMP1.14) has demonstrated a good anti-tumor activity in syngeneic models. This antibody, which is a rat IgG2a antibody, has been here reformatted into a mouse Fc-silent IgG1 format (MOS2) and by using a mouse syngeneic model (MC38=colon adenocarcinoma cells), its activity has been compared with the reference RMP1.14 clone (BioXcell).



Antibody Name	Doses tested (mpk=mg/kg)	Injections schedule	Mice/ group
MOS2-mPD1 (=FC Silent mouse IgG1)	12.5/2.5/1.25/0.5 IV	3 (D0;D3;D7)	8
MOS2-IC-1F3 (=FC Silent mouse IgG1)	12.5 IV	3 (D0;D3;D7)	8
a-mPD1 (=Fc competent rat IgG2a)	12.5/2.5/1.25/0.5 IV	3 (D0;D3;D7)	8
IC rat IgG2a (Clone 2A3; BioXcell)	12.5 IV	3 (D0;D3;D7)	8
Vehicle	IV	3 (D0;D3;D7)	8

Figure 3: **Experimental design for the comparison of two different anti-mPD1 antibodies in a mouse syngeneic model** : MOS2-mPD1 (= Fc silent mouse IgG1) versus anti-mPD1 (= Fc competent rat IgG2a, clone RMP1.14). IC, isotype control.

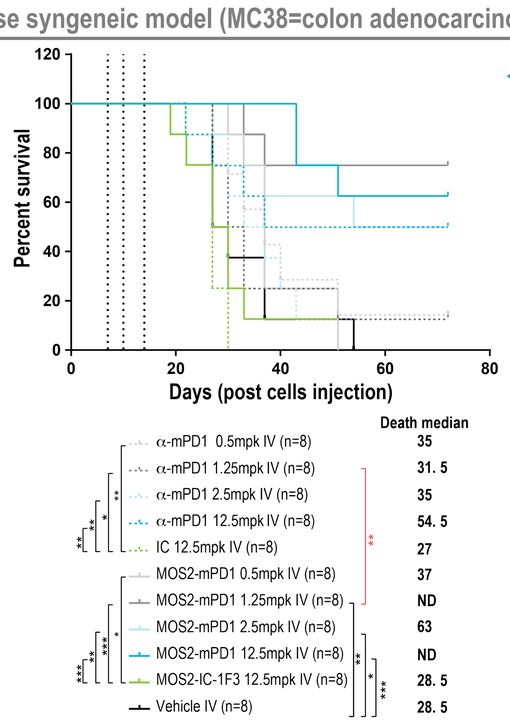


Figure 4: **Mouse survival follow-up.**

Using Mantel-Cox test, mouse survival is significantly better in groups treated with : 1) **MOS2-mPD1 mouse IgG1** compared to a-mPD1 rat IgG2a at 1.25 mpk; 2) a-mPD1 rat IgG2a or MOS2-mPD1 mouse IgG1 at all doses compared to IC groups; and 3) MOS2-mPD1 mouse IgG1 at 1.25/2.5/12.5 mpk compared to vehicle group. *, p<0.05; **, p<0.01; ***, p<0.001.

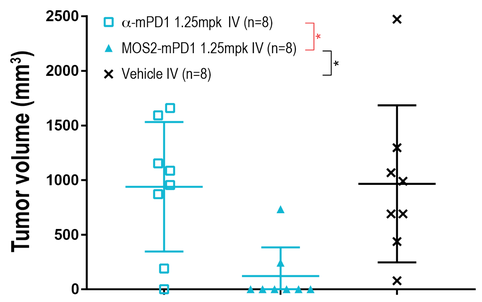


Figure 5: **Comparison of individual tumor volumes at day 27.**

Using the Dunn's test, we observed significant differences in tumor volume between MOS2-mPD1 mouse IgG1 compared to a-mPD1 rat IgG2a at 1.25 mpk. *, p<0.05.

- **As expected, MOS2-mPD1 mouse IgG1 and anti-mPD1 rat IgG2a treatment at 12.5mpk were efficient on MC38 model.**
- **At 1.25mpk, MOS2-mPD1 mouse IgG1 was more potent than the anti-mPD1 rat IgG2a treatment (lower tumor volumes, higher survival).**

→ This mouse Fc-silent version of the anti-mPD1 surrogate showed significantly better activity to the rat subclass. This may be related to the fact that no ADCC activity towards mice T cells and / or mouse immunization against the injected antibody should occur when using mouse Fc silent format (contrary to the Fc competent rat IgG2a format). Moreover, this mouse IgG1 format makes it possible to mimic the effect of the reference therapeutic antibody without causing the development of anti-species antibodies induced by rat antibodies in mouse syngeneic models. This surrogate format is therefore of valuable interest for preclinical studies.

As demonstrated here, MI-mAbs has extensive expertise in recombinant antibody engineering and generation, and can help you in the development of novel therapeutic antibodies. Starting from the advised choice of the appropriate formats, we can integrate the engineering, production and purification of recombinant antibodies and, finally, the pharmacological validation for the proof of concept *in vitro* and *in vivo*.

Bibliography:
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