#2665

Monday April 8, 2024
1:30 PM - 5:00 PM

Fc silencing of commonly used surrogate anti-PD-1 RMP1.14 increases its therapeutic profile in MC38 syngeneic model

ESTER MORGADO, LAURENT POUYET, ANAIS JOACHIM, OLIVIER DEAS, FRANÇOIS ROMAGNE

MImAbs, 117 Avenue de Luminy, 13009 Marseille, France | contact@mimabs.org

Introduction

Anti-PD-1 treatment has become a backbone treatment in several cancers such as melanoma and lung carcinoma. Adding new drugs to PD-1 requires careful evaluation of combination treatments in preclinical models to evaluate efficacy and tolerability. The syngeneic tumor model remains one of the best ways of preclinical evaluation of combinations, as such evaluation requires fully immune competent animals to correctly assess both the PD-1 contribution and the added value or synergistic effect of new drugs in the combination. Anti mouse PD-1 such as RMP1.14, a rat IgG2a, is widely used in these models as an anti-PD-1 mAb surrogate for such studies. Although RMP1.14 demonstrated efficacy in several models such as MC38 and CT26, it may not fully recapitulate the use of blocking mAb in humans as it may be immunogenic in mice and may retain some agonist activity due to its rat isotype. Here, we demonstrate that engineering this antibody with a mice Fc silent backbone increases its activity in the MC38 model.

Methods

VH/VL from RMP1.14 rat IgG2a (a-mPD-1) were sequenced and engineered with a mouse Fc silent IgG1 backbone (N297Q) and produced in CHO. Both antibodies (a-mPD-1: rat IgG2a ; and MOS2-mPD-1: Fc silent mouse IgG1) were purified on protein A using standard procedures (upper panel).
MC38 (1 million cells) were injected subcutaneously in C57BL/6 mice, and randomized when tumor volume reached 100 mm3. Dose effect of a-mPD-1 and MOS2-mPD-1 and their corresponding isotype controls were tested in this MC38 model at doses of 0.5 ; 1.25 ; 2.5 and 12.5 mg/kg at day 0, day 3, and day 7 post randomization (8 mice per group, fig 1). Treatment route effect was tested in the same model by comparing intraperitoneal and intravenous injections in an additional experiment using both antibodies at 1.25 mg/kg and 12.5 mg/kg doses (fig 5).

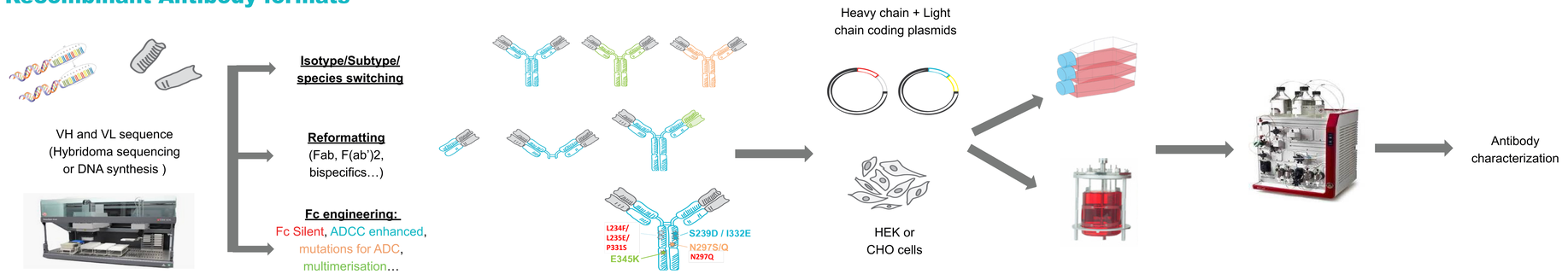
Results

Both a-mPD-1 and MOS2-mPD-1 demonstrated efficacy in survival in MC38 model at all tested doses (p<0,05 Mantel-Cox test) compared to their isotype controls (fig 2). MOS2-mPD-1 demonstrated increased efficacy in terms of complete responses (fig 4 and 8) in the above escalated doses (CR of 12.5, 12.5, 12.5 and 37.5 % and 0, 75, 50 and 62.5 % for a-mPD-1 and MOS2-mPD-1 respectively). Tumor volumes and survival were also always lower in the MOS2-mPD-1 groups compared to dose corresponding a-mPD-1 groups and were statistically different in two independent experiments at medium doses (P<0.05 Dunn's test performed at 1.25mg/kg at day 27, and Mantel-Cox test, fig 3 and 7). IP and IV routes were equivalent in efficacy at the tested doses.

Conclusion

Mouse Fc engineered surrogates have a significantly different behavior in mouse models compared to the commonly used surrogates usually from rat origin. These new formatted antibodies should better mimic the efficacy and tolerability of antibodies used in humans, and should be preferred when new combinations of treatments are tested.

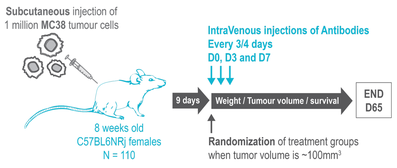
Recombinant Antibody formats



In vivo study of "reformatted"anti-PD-1 surrogate: Dose effect of Fc silent (MOS2-mPD-1) and Rat anti mouse PD-1 (a-mPD-1) via IV injection

In preclinical studies, the widely used anti-mouse PD-1 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). Its activity has been compared with the reference RMP1.14 clone from BioXcell (a-mPD1) by using a mouse syngeneic model (MC38=colon adenocarcinoma cells).

Figure 1: Experimental design for the comparison of two different anti-mPD-1 antibodies in a mouse syngeneic model: MOS2-mPD-1 (= Fc silent mouse IgG1) versus a-mPD-1 (= Fc competent rat IgG2a, clone RMP1.14). IC, isotype control.



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

Figure 3: Comparison of individual tumor volumes at day 27.
Using the Dunn's test, we observed significant differences in tumor volume between MOS2-mPD-1 mouse IgG1 compared to a-mPD-1 rat IgG2a at 1.25 mpk. *, p<0.05.

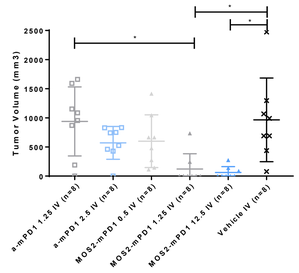
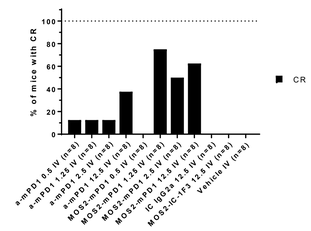


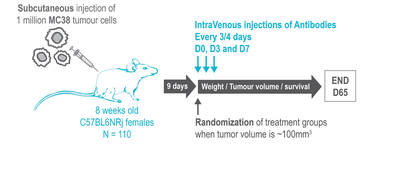
Figure 4: Comparison of complete response rates.
At the end of treatment period, mice experiencing Complete tumor Regression (CR) occurred more frequently in MOS2-mPD-1 mouse compared to a-mPD-1



MOS2-mPD-1 and a-mPD-1 surrogate: Dose effect comparing Intravenous (IV) and Intraperitoneal (IP) injections

In order to confirm the above data, a second independent experiment was performed with a similar design, but also comparing two widely used injection routes for the two mAbs.

Figure 5: Experimental design for the comparison of two different anti-mPD1 antibodies in a mouse syngeneic model: MOS2-mPD-1 (= Fc silent mouse IgG1) versus a-mPD-1 (= Fc competent rat IgG2a, clone RMP1.14). IC, isotype control.



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

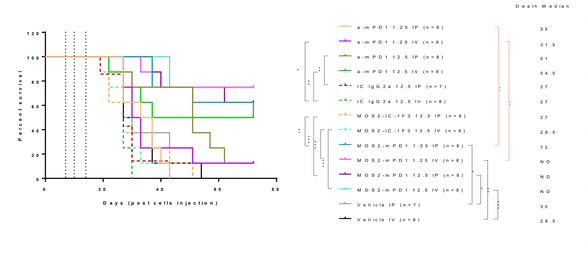


Figure 6: Mouse survival follow-up.
Using Mantel-Cox test, mouse survival is significantly better in groups treated with: 1) MOS2-mPD-1 mouse IgG1 compared to a-mPD-1 rat IgG2a at 1.25 mpk both with IP or IV routes 2) a-mPD-1 rat IgG2a or MOS2-mPD-1 mouse IgG1 at all doses compared to IC groups except 1,25 IP route and 3) MOS2-mPD-1 mouse IgG1 at 1.25/12.5 mpk compared to vehicle group both with IP or IV routes. *, p<0.05; **, p<0.01; ***, p<0.001.

Figure 7: Comparison of individual tumor volumes at day 27.
Using the Dunn's test, we observed significant differences in tumor volume between MOS2-mPD-1 mouse IgG1 compared to a-mPD-1 rat IgG2a at 1.25 mpk, whatever the IP or IV injection route. *, p<0.05.

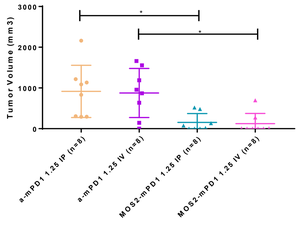
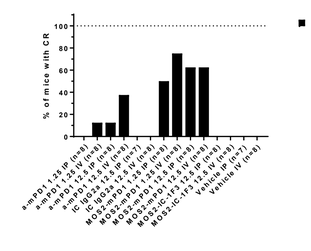


Figure 8: Comparison of complete response rates.
At the end of treatment period, mice experiencing Complete tumor Regression (CR) occurred more frequently in MOS2-mPD-1 mouse compared to a-mPD-1



Bibliography:
G. A. Lazar et al. Engineered antibody Fc variants with enhanced effector function. (2006) PNAS, 103: 4005-4010 / C. W. Lin et al. A common glycan structure of immunoglobulin G for enhancement of effector functions. (2015) PNAS, 112:10611-10616.