

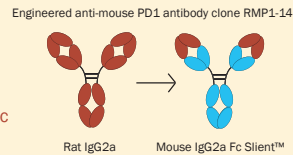
# Consistent, long-term PD1 blockade using a syngeneic, engineered anti-mPD1 antibody: Superior tools for consistent and more meaningful immunotherapy research.



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Antibodies targeting immune checkpoint proteins are becoming an important branch of anti-cancer therapeutics. In order to further our understanding of the mechanisms underlying mono- as well as combination-therapies, new research tools are required as widely used research reagents are not benefiting from the same protein engineering advances therapeutics can harness. The majority of antibodies used *in vivo* in mice are of rat or hamster origin, and are therefore immunogenic, leading to adverse immunological reactions and gradual loss of activity.

In the following we sought to compare short-term and long-term efficacy of the original rat IgG2a version of the anti-mouse PD1 antibody RMP1-14 against a recombinantly engineered version of RMP1-14 featuring an Fc Silent™ syngeneic mouse IgG2a Fc domain in a non-immunogenic HEP1-6 liver cancer model.

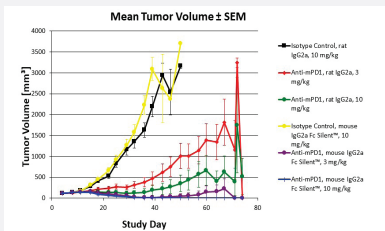


## METHODS

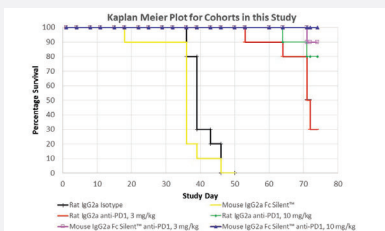
Low endotoxin <0.1 EU/mg, recombinant versions of the anti-mouse PD1 antibody RMP1-14 (Ab00813-7.1, Ab00813-2.3) and the corresponding isotype control anti-fluorescein antibody 4-20-20 (Ab00102-7.1, Ab00102-2.3) were produced by Absolute Antibody Ltd. Female BALB/c mice were inoculated with *in vitro* propagated HEP1-6 murine liver cancer cells, and treatment commenced after tumors had grown to >100 mm<sup>3</sup> after 4 days. Humane end-points were set to 3000 mm<sup>3</sup> for each animal. Animals were treated biweekly with either 3 mg/kg or 10 mg/kg I.P. injections of anti-PD1 antibodies or 10 mg/kg of isotype control antibody.

## RESULTS

Syngeneic mouse IgG2a Fc Silent™ anti-PD1 antibodies are more potent than rat IgG2a anti-PD1 antibodies.



Mean tumor sizes in animals treated with 3 mg/kg mouse IgG2a Fc Silent™ are significantly smaller than in animals treated with the higher 10 mg/kg dose of rat IgG2a antibody. Moreover, gradual increase of mean tumor size is seen amongst animals treated with the rat IgG2a version.



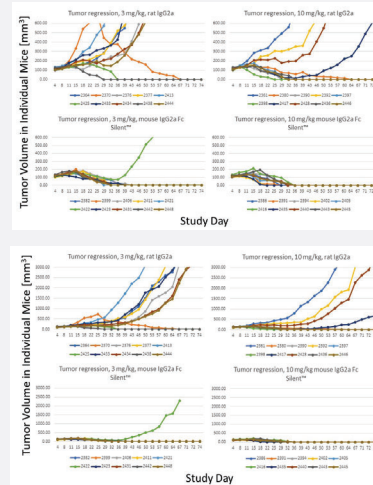
Differences in mortality between the formats of the anti-mPD1 antibody are only seen at later stages of the study.

## CONCLUSIONS

- Engineered, syngeneic antibodies are more potent, requiring smaller injections compared to their parental format.
- More homogenous and robust treatment responses allow for more representative and meaningful data even with smaller cohorts.
- In order to obtain experimental results unaffected by systemic immunological responses to foreign antigen, syngeneic antibodies should be used for all *in vivo* applications.

## RESULTS

Treatment with syngeneic anti-PD1 antibody results in a more consistent treatment response.



Short-term responses to treatment with the mouse IgG2a Fc Silent™ antibody show considerably less variation than those treated with the rat IgG2a version.

10/10 animals treated at 10 mg/kg and 9/10 animals treated at 3 mg/kg of the mouse IgG2a Fc Silent™ completely cleared the tumor.

Only 3/10 animals treated with 3 mg/kg and 6/10 animals treated with 10 mg/kg of the rat IgG2a antibody fully rejected the tumor. Once cleared, no animals relapsed upon cessation of treatment.

## DISCUSSION

We used a non-immunogenic, murine HEP1-6 liver cancer model to show that engineered, syngeneic mouse IgG2a Fc Silent™ antibodies show superior potency compared to the original rat IgG2a antibody. Amongst the animals that responded to the treatment, full tumor rejection had set in between D29 and D43; however, even at 10 mg/kg of rat IgG2a, only 5/10 animals responded in that time frame, with 1 late responder and 4 non-responders. At 3 mg/kg of mouse IgG2a Fc Silent™ 9/10 animals had rejected the tumor in that time frame, with one animal showing no long-term protective response. All animals treated with 10 mg/kg IgG2a Fc Silent™ fully cleared the tumor.

We propose that the superior activity of the mouse IgG2a Fc Silent™ antibody is due to its lack of immunogenicity in mice, compared to the rat IgG2a antibody. Whilst primary immune responses lead to an initial heterogeneity in the early response to treatment, the onset of a robust secondary immune response post D28 leads to strong neutralization of the injected antibody and results in tumor out-growth.

As the syngeneic antibodies used still contained antigen-binding domains of rat origin, some residual immunogenicity, leading to anti-idiotypic antibodies, can explain the out-growth seen with one animal in the cohort treated with 3 mg/kg mouse IgG2a Fc Silent™; however, the low frequency of this occurrence strongly supports the utility of these chimeric reagents.

The more homogenous early response to the antibody treatment seen with the mouse antibody compared to the rat antibody-treated cohort suggests that fewer animals may be used in early-response studies and still yield representative and meaningful data.

These data also highlight that current experimental protocols using antibodies that are not matched to their target species risk leading to inaccurate results, especially in the investigation of immune-responses. This is due to all observations being made on the background of a systemic immune-response to repeatedly injected foreign antigen. We therefore propose, that where at all possible, syngeneic antibodies should be used for all *in vivo* experiments.