Establishment of an *in vivo* mouse model to study and overcome infusion related reactions associated with FcγRIIB antibody administration

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Introduction

Administration of therapeutic monoclonal antibodies (mAb) is often accompanied by infusion related reactions (IRR), of ill-determined mechanism. During the clinical development of BI-1206 (NCT02933320 and NCT03571568), a fully human mAb that binds specifically to hFcγRIIB (hCD32B), frequent IRR were observed, independent of sustained FcγRIIB blockade. At 100mg/patient, BI-1206 showed transient receptor saturation for up to 72h, whereas IRR most often resolved within 24h. Administration of BI-1206 was also associated with transient thrombocytopenia but not increased bleeding, and most episodes resolved within a week. Elevated transaminases (i.e. alanine transaminase (ALT) and aspartate transaminase (AST)) and a transient cytokine release was also observed alongside thrombocytopenia.

Methods

Six to eight-week-old C57BL/6 mice were injected either through intravenous (i.v.), intra-peritoneal (i.p.) or subcutaneous (s.c.) routes with a surrogate mouse anti-FcγRIIB (AT-130-2 IgG2a) in doses ranging from 1-400μg/mouse. Mice were monitored post injection for changes in behavior and macroscopic symptoms such as isolation, mobility, and fur condition.

Serum concentrations of AT130-2 were quantified using an in-house ELISA. Platelet counts were analyzed in fresh blood using a Vetscan (Triolab). Transaminases were analyzed by IDEXX BioResearch. Cytokines were analyzed with the V-plex Proinflammatory Panel 1 Mouse kit (MesoScale Discovery), including interferon (IFN)-γ, interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, KC/GRO and tumor necrosis factor (TNF)-α.

Results

Following administration of 200 μg AT130-2 i.v., rapid onset of IRR was seen within 5-7 minutes. Blood sampling of these mice indicated reduced blood pressure. The mice recovered 10-15 minutes post IRR onset and no macroscopic symptoms were observed by 1 h. Tolerability was dose-dependent and showed a clear pattern of increasing IRR with regards route of administration in the order of s.c. < i.p. < i.v. When comparing PK and receptor occupancy with the onset, severity and duration of IRR, there was a clear correlation between high dose and rapid increase in serum concentration, rather than FcγRIIB saturation.

A decrease in platelet count was seen at the same time as IRRs after both the i.v. and i.p. administration. The decrease was transient and normalized within 8h post injection. AST, ALT, IL-5, IL-6, IL-10, KC/GRO, TNF-α showed a transient increase, all peaking 1-3h post injection, except for IL-5. IL-5 showed a delayed peak 3-8h post injection.

To investigate whether premedication with corticosteroids could inhibit the IRR and associated toxicities, mice were premedicated with betamethasone or dexamethasone 16-24h s.c. and 1h i.v. pre injection of AT-130-2. Development of IRR and platelet reduction was completely inhibited with premedication. Also, the increase in liver transaminases and cytokine release was significantly reduced. A single dose of premedication at 1h pre injection did not inhibit the IRR nor prevent the decrease in platelet count. Whilst a single dose of premedication 16-24h pre injection reduced the IRR and platelet decrease, it did not completely block the changes, indicating that two doses of corticosteroids are optimal.

Conclusions

Here we demonstrate an *in vivo* model that recapitulates the tolerability profile seen with BI-1206 in human cancer subjects, including IRR, decreased platelet count, elevated transaminases and transient cytokine release. In the mouse model, there was a correlation between IRR with high dose and rapid increase in serum concentration, rather than FcγRIIB saturation. Pre-treatment with two doses (16-24h and 1h) of corticosteroids appears imperative to enable complete and sustained FcγRIIB saturation following administration of anti-FcγRIIB without IRR. This pre-medication regimen has been implemented in clinical trials and shown to improve tolerability to BI-1206.