Afimetoran, a potent and selective inhibitor of human Toll-like receptor 7 (TLR 7) and TLR 8, provides robust efficacy in a murine lupus model of advanced disease

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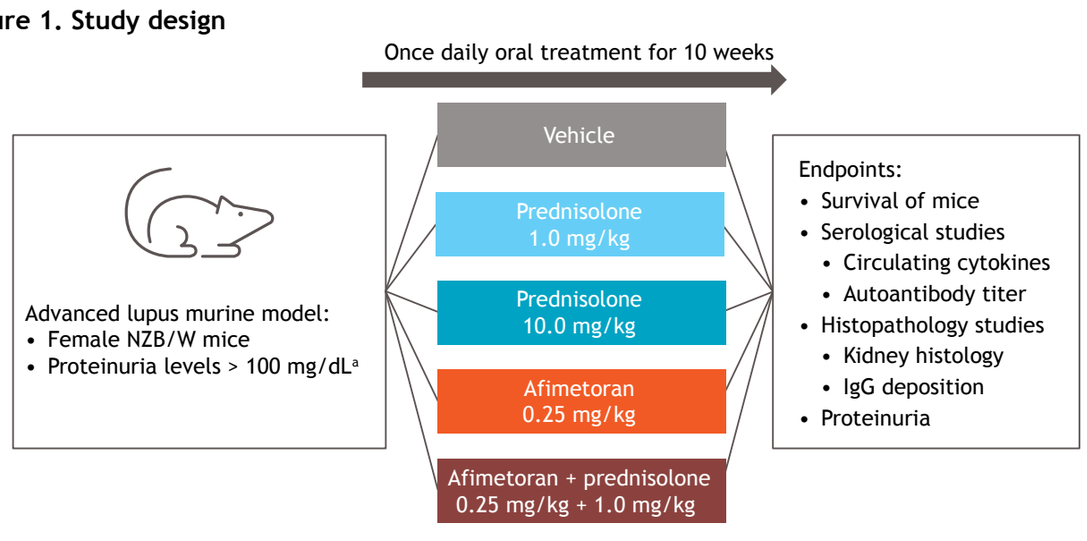
Background

- Toll-like receptors 7 and 8 (TLR 7/8) recognize single-strand ribonucleic acid (ssRNA) from pathogens and self-apoptotic sources[1,2]
- The autoimmune disease lupus erythematosus is associated with defects in apoptotic debris clearance that can activate TLR 7/8, which are implicated in the initiation and progression of disease[2-5]
- Activation of TLR 7/8 in multiple immune cell types leads to a pathological immune response with elevated levels of pro-inflammatory cytokines, including type I/III interferons, and steroid resistance[2,6-9]
- Afimetoran (BMS-986256) is a potent, selective inhibitor of TLR 7/8 that has shown robust efficacy in vitro and in vivo[10,11]
  - Afimetoran inhibits the production of interleukin 6 and interferon alpha in a dose-dependent manner in mice challenged with the TLR 7 agonist gardiquimod (mice lack a functional TLR 8)[10]
- We present the therapeutic efficacy of afimetoran in a murine model of advanced lupus

Methods

- Female New Zealand Black/White hybrid (NZB/W) mice with proteinuria > 100 mg/dL (ie, advanced disease) were treated orally for 10 weeks with vehicle (negative control), prednisolone (positive control), afimetoran, or a combination of afimetoran and low-dose prednisolone (**Figure 1**)
- The effects of treatment on disease severity and survival were assessed

Figure 1. Study design



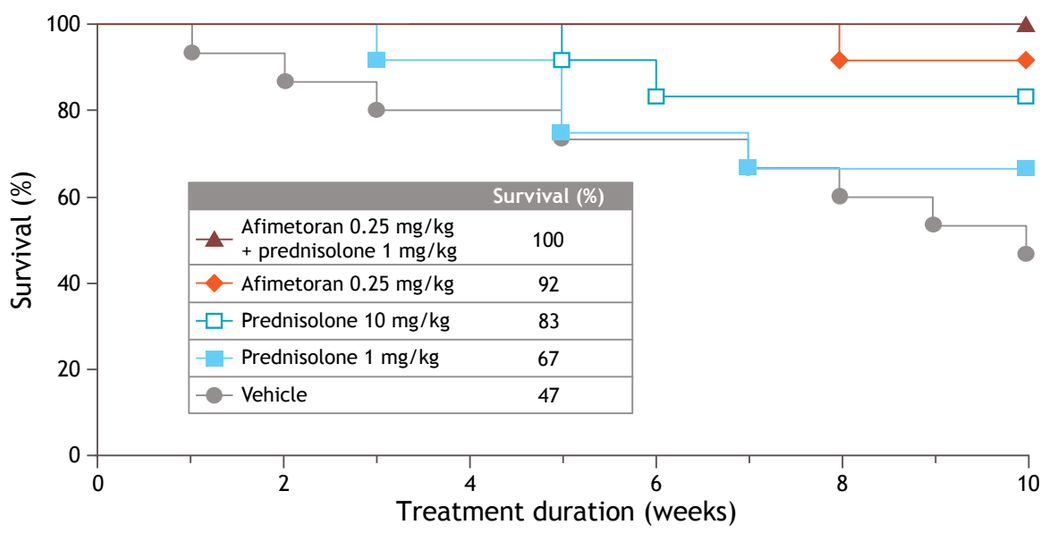
[a]Urine protein levels in normal NZB/W mice without any disease are < 30 mg/dL. IgG, immunoglobulin G.

Results

Survival rates in mice with advanced lupus

- Afimetoran improved survival of the NZB/W murine model of advanced lupus (**Figure 2**)
- Treatment with afimetoran alone or in combination with low-dose prednisolone resulted in survival rates of 92% and 100%, respectively; in contrast, treatment with vehicle resulted in 47% survival

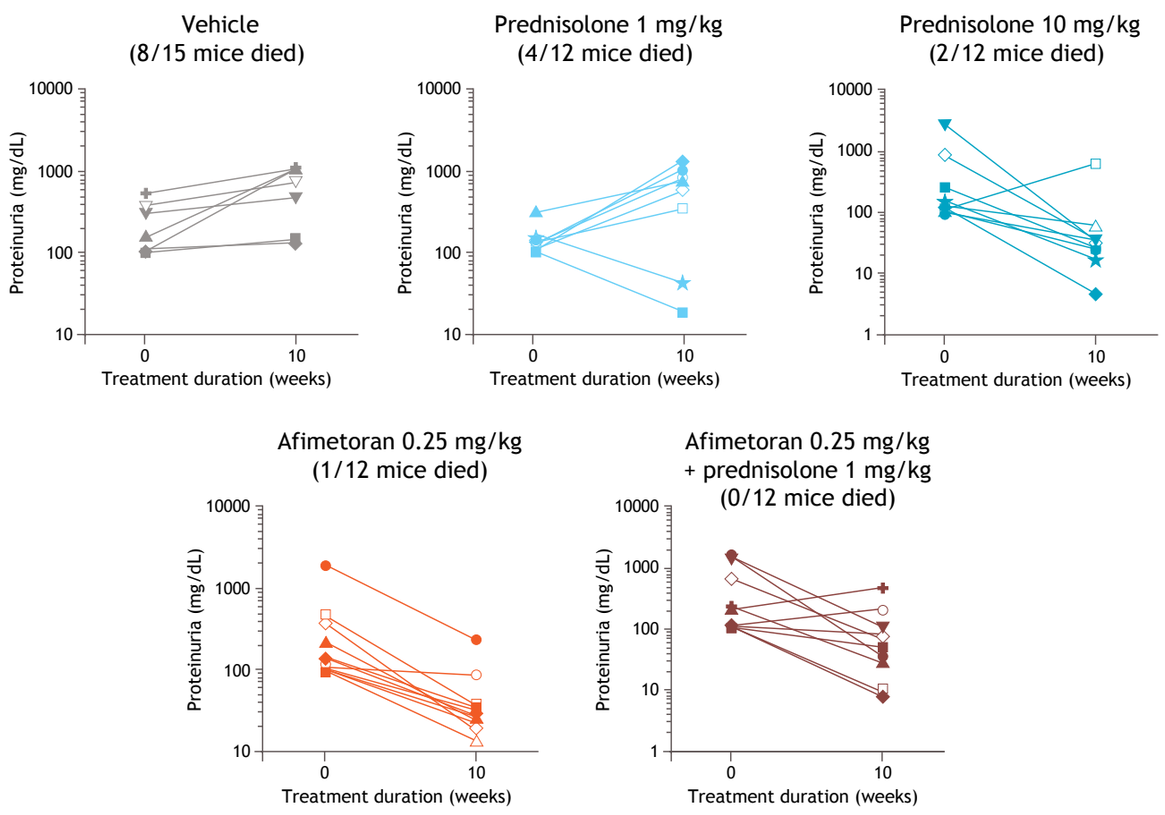
Figure 2. Afimetoran improved survival of the NZB/W murine model of advanced lupus



Renal function in mice with advanced lupus

- Treatment with 0.25 mg/kg afimetoran reversed proteinuria to normal levels in the majority of animals, in contrast to treatment with low-dose (1 mg/kg) prednisolone (**Figure 3**)
- Overall, afimetoran 0.25 mg/kg resulted in a 92% reduction in proteinuria as compared with the surviving members of the vehicle group

Figure 3. Afimetoran reverses proteinuria in the NZB/W murine model of advanced lupus

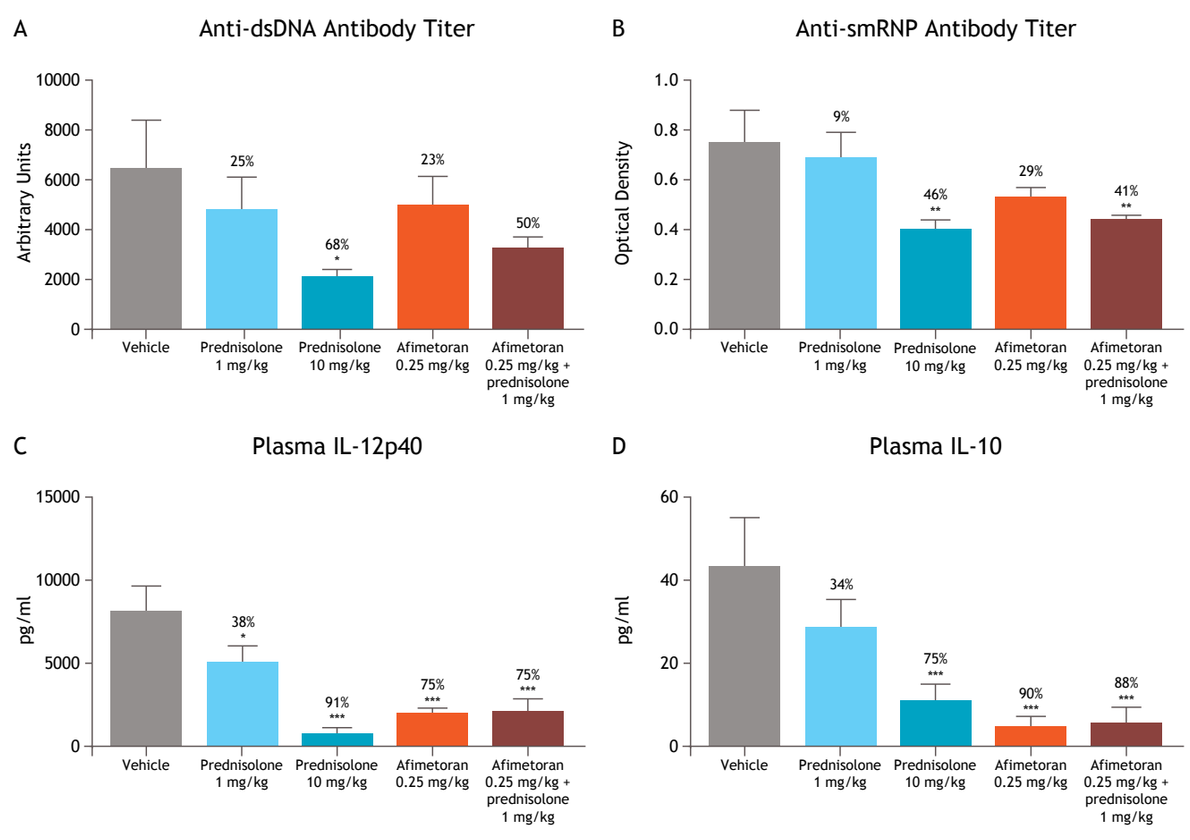


Change in proteinuria levels during the course of the study for individual mice are indicated by separate symbols in each group. Proteinuria in normal NZB/W mice is < 30 mg/dL.

Circulating autoantibody and cytokine levels

- Afimetoran 0.25 mg/kg + low-dose prednisolone inhibited autoantibody titers by 50% and 41% for double-stranded DNA (dsDNA) and small nuclear ribonucleoprotein (smRNP), respectively, relative to mice administered vehicle alone (**Figure 4A, B**)
- Afimetoran 0.25 mg/kg reduced levels of the circulating cytokines IL-12p40 and IL-10 by 75% and 90%, respectively, relative to mice administered vehicle alone (**Figure 4C, D**)

Figure 4. Afimetoran lowered levels of autoantibodies and significantly reduced levels of circulating cytokines in the NZB/W murine model of advanced lupus

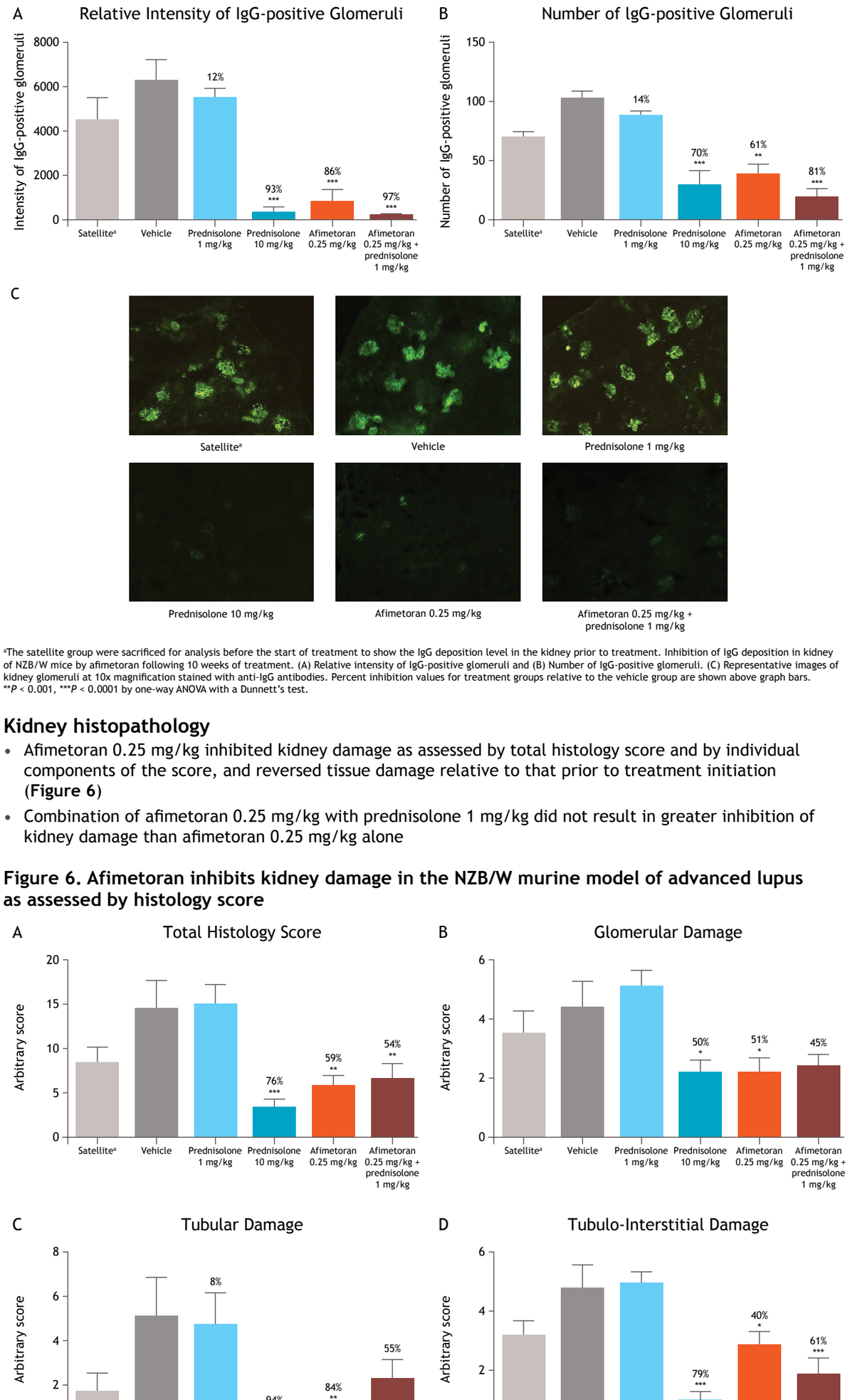


Following 10 weeks of dosing, multiple biomarkers were assessed, including (A) Serum anti-dsDNA antibody titer, (B) Anti-smRNP antibody titer, (C) Plasma levels of IL-12p40, and (D) Plasma levels of IL-10. Data are from one experiment with 7 to 12 mice per group. Percent inhibition values for treatment groups relative to the vehicle group are shown above graph bars. *P < 0.01, **P < 0.001, ***P < 0.0001 by one-way ANOVA with a Dunnett's test.
ANOVA, analysis of variance; IL-10, interleukin 10; IL-12p40, interleukin 12 protein 40.

Glomerular IgG deposition

- Treatment with afimetoran inhibited renal glomerular IgG deposition (**Figure 5**)
- After 10 weeks, there was a reduction in the intensity and number (86% and 61%, respectively, relative to the vehicle group) of IgG-positive glomeruli in mice treated with afimetoran 0.25 mg/kg, reducing IgG deposition to levels below those seen at the start of treatment in the satellite group

Figure 5. Afimetoran inhibits glomerular IgG deposition in the NZB/W murine model of advanced lupus



[a]The satellite group were sacrificed for analysis before the start of treatment to show the IgG deposition level in the kidney prior to treatment. Inhibition of IgG deposition in kidney of NZB/W mice by afimetoran following 10 weeks of treatment. (A) Relative intensity of IgG-positive glomeruli and (B) Number of IgG-positive glomeruli. (C) Representative images of kidney glomeruli at 10x magnification stained with anti-IgG antibodies. Percent inhibition values for treatment groups relative to the vehicle group are shown above graph bars. **P < 0.001, ***P < 0.0001 by one-way ANOVA with a Dunnett's test.

Kidney histopathology

- Afimetoran 0.25 mg/kg inhibited kidney damage as assessed by total histology score and by individual components of the score, and reversed tissue damage relative to that prior to treatment initiation (**Figure 6**)
- Combination of afimetoran 0.25 mg/kg with prednisolone 1 mg/kg did not result in greater inhibition of kidney damage than afimetoran 0.25 mg/kg alone

Figure 6. Afimetoran inhibits kidney damage in the NZB/W murine model of advanced lupus as assessed by histology score

[a]The satellite group indicates histopathology scores before the initiation of treatment. Following 10 weeks of treatment, samples were collected and histopathology analysis was performed. Each sample was scored on a scale of 0-4 as follows: 0=unremarkable changes; 1=minimal; 2=mild; 3=moderate; and 4=marked change. The total score is the sum of 9 categories and the theoretical maximum score is 36. (A) Total histology score. (B) Glomerular damage. (C) Tubular damage. (D) Tubulo-interstitial damage. Percent inhibition values for treatment groups relative to the vehicle group are shown above graph bars. *P < 0.05, **P < 0.01, ***P < 0.001, by one-way ANOVA with Dunnett's test.

Conclusions

- Afimetoran, a highly potent and selective inhibitor of TLR 7/8, displayed robust efficacy in an NZB/W murine model of advanced lupus
- Afimetoran treatment, including in combination with low-dose prednisolone, led to:
  - Improved survival
  - Reversal of proteinuria and glomerular IgG deposition
  - Reduction in circulating cytokine levels
- Afimetoran may offer a novel therapeutic approach for the treatment of active systemic lupus erythematosus and is currently being investigated in a phase 2 clinical trial (NCT04895696)[12]

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