PHARMACOKINETIC AND TOXICITY STUDIES OF AN ANTI-CD40LG ANTIBODY AT-1501 IN RHESUS MACAQUES

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Introduction

CD40LG, a costimulatory receptor for CD40 is found on T helper cells. Binding of CD40LG on activated T cells to CD40 expressed on antigen presenting cells induces downstream immune and inflammatory responses. Inhibition of CD40LG signaling can abolish inflammation, induce T cell anergy, prevent the progression of autoimmunity and instill transplant tolerance. AT-1501 anti-CD40LG is a humanized IgG1 antibody modified to remove Fc effector function with high-affinity binding to CD40LG.

A single dose pharmacokinetic study of AT-1501 given intravenously (IV) at 1, 10, or 50 mg/kg demonstrated an elimination half-life of 7-9 days. Half-life variability at the lower doses was attributed to immunogenicity of AT-1501 in rhesus macaques. Plasma concentrations contributing to AUC were affected by the induction of a neutralizing anti-drug antibody (ADA) response which occurred markedly at 1 mg/kg, to an intermediate extent at 10 mg/kg, and was absent at 50 mg/kg.

We conducted 12-week (3, 10, 30, 50 mg/kg) and 26-week (100, 200 mg/kg) toxicology studies using weekly IV dosing of AT-1501 in rhesus macaques. Immunogenicity was observed at 3 mg/kg. Half of the 10 mg/kg animals also showed ADA responses, but these were non-neutralizing and did not impact the levels of circulating AT-1501. Neutralizing ADA responses were not observed in doses above 10 mg/kg. All animals in the 100 mg/kg dose group completed the 26-week study, remained healthy and achieved predicted AT-1501 concentrations in plasma. All animals in the 200 mg/kg group completed 16 weeks without evidence of toxicity. Four of the 200 mg/kg animals showed signs of toxicity and were euthanized at 17, 18, 25 and 26 weeks, respectively. Data from these animals suggested a Type III hypersensitivity infusion reaction (IR) resulting in immune-complex (IC) formation, impacting multiple organs, but primarily resulting in kidney pathology and dysfunction. In addition to significantly reduced plasma Complement CH50 levels, clinical chemistry and histopathology confirmed extravascular localization of AT-1501, NHP IgG1 and IgM, albumin and Complement C3. These results support the conclusion that the toxicity was due to a high concentration of circulating AT-1501 leading to IC formation and not due to a direct action of the drug. ICs were comprised of NHP IgM anti-AT1501, demonstrating that AT-1501 effectively blocked IgM to IgG class switching. These data demonstrate the immune modulating activity of AT-1501 in vivo. Thus, weekly intravenous 10 mg/kg AT-1501 doses are considered sufficient to inhibit antigen presentation and pro-inflammatory lymphocyte signaling.

Critical Objectives

(1) Assess pharmacokinetic parameters of AT-1501 in nonhuman primates (Figure 1).

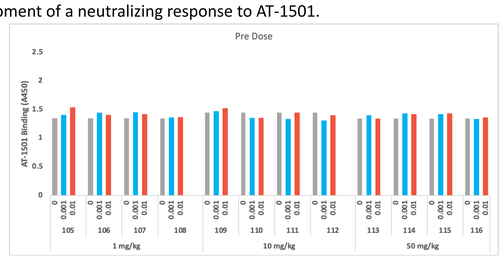
(2) Assess anti-drug antibody responses and potential neutralizing activity to AT-1501 in nonhuman primates (Figure 2).

(1) Conduct chronic repeated-dose toxicity studies in nonhuman primates over a range of dose levels to assess changes in hematology (Figure 3, Table 1), clinical chemistry, body weight, body temperature, blood pressure, ocular, neurological, cardiac, and respiratory function and to reveal any gross or microscopic toxicity.

Methodology

Figure 1. AT-1501 Concentration over Time in Rhesus Monkey Plasma Samples. An ELISA was used to quantitate the levels of circulating AT-1501 in Rhesus monkey plasma samples following a single intravenous bolus of AT-1501 at 0, 1, 10, or 50 mg/kg. 96-well plates were coated with recombinant human CD40LG, plates were blocked with PBS/BSA to prevent non-specific background binding, dilutions of Rhesus plasma samples in assay buffer along with a standard curve of AT-1501 were added to plates, AT-1501 bound to CD40LG was detected with HRP-(Fab'2) donkey anti-human IgG antibody, subsequent incubation with colorimetric HRP substrate, and read on a SpectraMax M5 plate reader to determine absorbance at 450 nm. Levels of AT-1501 in each sample were determined from the standard curve of AT-1501 run on each plate.

Figure 2: Determination of Neutralizing Rhesus Anti-AT-1501 Antibody Response A competition ELISA assay was used to determine if the polyclonal Rhesus anti-AT-1501 antibody response to AT-1501 was neutralizing. 96-well plates were coated with recombinant human CD40LG and blocked with PBS/BSA to prevent non-specific background binding. Dilutions of the pre-dose, 1008hr PK plasma samples or assay buffer were pre-incubated with AT-1501 and then transferred to the CD40LG coated plate. AT-1501 bound to CD40LG was detected with HRP-(Fab2) donkey anti-human IgG antibody and incubation with colorimetric HRP substrate. Absorbance at 450nm was determined. Samples were positive if A450 of plasma/AT-1501 mix was lower than A450 of buffer/AT-1501 mix . Neutralizing responses occurred after a single dose of 1mg/kg or 10mg/kg, but not with 50mg/kg, AT-1501. Repeat dose studies demonstrated that weekly dosing of AT-1501 at 10mg/kg or higher prevented the development of a neutralizing response to AT-1501.



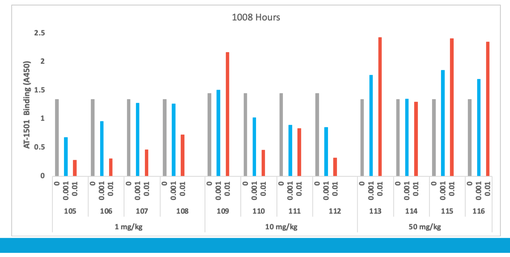


Figure 3. Hematology Differential Cell Count Analysis. The individual animal data for all dose cohorts (0, 3, 10, 30, 50, 100, 200 mg/kg) sampled during the 12 and 26 week toxicity studies were compiled. Individual box plots for each parameter are plotted irrespective of sample time.

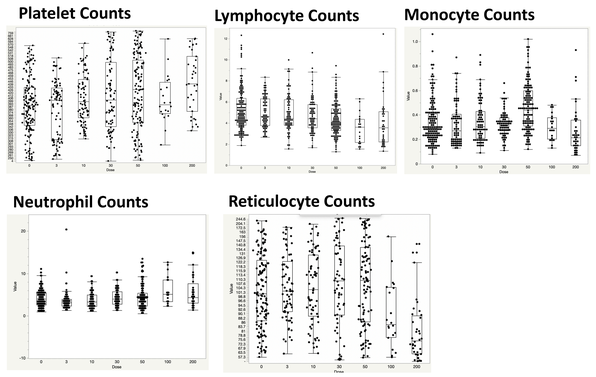


Table 1. Statistical Analysis of Hematology Data. Dunnett's test was used to determine whether any dose group means were different from the control mean (dose = 0) after a one-way analysis of variance (ANOVA). This test controls the significance level for multiple comparisons. P-values shown in the table are those derived by the Dunnett's test when comparing whether counts in each dose group were statistically significantly ($p<0.05$) different from the control (dose = 0) dose group.

		DOSE (mg/kg)					
Parameter	**Day**	**3**	**10**	**30**	**50**	**100**	**200**
BAS	28	0.6547	1.0000	0.2839	0.5670	0.1690	0.0742
	84	0.9963	1.0000	0.9694	0.6515		
	126					0.9851	0.7054
EOS	28	0.9994	0.7304	0.6652	0.9959	0.7922	0.8805
	84	0.9993	0.5731	0.9977	1.0000		
	126					0.5128	0.9757
LYM	28	0.7219	0.9619	0.3806	0.2395	0.0062	0.0221
	84	0.8912	0.9122	0.9998	0.9990		
	126					0.4895	0.7751
MON	28	0.5702	0.9542	0.2821	0.9998	0.9994	0.9980
	84	0.9731	0.9342	0.9164	0.0170		
	126					0.1484	0.5852
NEU	28	0.2095	0.7093	0.9988	0.9544	0.7759	0.9813
	84	0.9358	0.9926	0.9997	0.9264		
	126					0.7941	0.8357
RETIC	28	0.6159	0.9916	1.0000	0.8105	0.5870	0.9224
	84	0.9991	0.6669	0.2738	0.8711		
	126					0.421	0.7256
PLT	28	0.9865	1.0000	0.7579	0.9919	0.7715	0.2439
	84	0.9363	1.0000	1.0000	0.2354		
	126					0.0719	0.1697

Results

- Dose proportionality was observed over the entire dose range during week 1, and also for the three higher dose levels (10, 30, 50 mg/kg), but not the lowest dose level (3 mg/kg) during Week 12, likely due to an ADA response.
- All of the 3 mg/kg/week group showed a neutralizing ADA response. Three of six animals in the 10 mg/kg dose group also showed a positive ADA response, but this response was not neutralizing. No ADA response was apparent at doses greater than 10 mg/kg/week.
- There were no differences in clinical chemistry, body weight, body temperature, blood pressure, ocular, neurological, cardiac, or respiratory function in any cohort.
- There were no changes in organ weights in animals receiving doses up to 100 mg/kg. There was a decrease in thymus and adrenal gland weights at doses of 100 and 200 mg/kg that likely contributed to decreased lymphocyte counts.
- Four animals in the 200 mg/kg cohort showed signs of dehydration, decreased activity, and watery feces. These animals were euthanized at weeks 17, 18, 25, and 26 respectively.
- Clinical chemistry and urine analysis showed decreased RBCs in circulation and proteinuria suggestive of delayed immune-complex (IC) -mediated infusion reaction (IR), caused by a rhesus immune complex-associated response to the humanized AT-1501 antibody.
- Clinical chemistry and histopathology in euthanized animals confirmed complement activation in circulation, localization of AT-1501, NHP IgG1, IgM, albumin, and Complement C3, intravascularly, and deposition in proximal and distal tubular epithelial intracellular inclusions, tubular lumens, in the glomerular urinary space, extra-vascularly in the interstitial tissue of the medulla, and in the interstitial tissue of the renal papilla.

Conclusion

- Weekly dosing of AT-1501 for 26 weeks in rhesus macaques was well tolerated and did not result in adverse findings at any dose level through 100 mg/kg.
- Dosing of AT-1501 at 200 mg/kg elicited a type III hypersensitivity infusion reaction (IR) with an ADA response resulting in immune complex (IC) formation impacting multiple organs but primarily resulting in kidney pathology and dysfunction. The delayed IR occurring after four months of dosing suggests that it is not IgE mediated (Type I hypersensitivity reaction).
- Immune complexes typically localize in small postcapillary venules (e.g., choroid plexus, ciliary body, synovium, and glomeruli) (Leach, 2014).
- The toxicity observed in the 200 mg/kg dose group resulted from the high concentration of circulating AT-1501 that lead to IC formation and was not due to the mechanism of action of the drug.
- Animal models tend to have low predictive value and often over-estimate immunogenicity rates and incidence of adverse immune-mediated events in human subjects (Wierda, 2001; Bugelski, 2004).
- Between 2004 and 2016, of the 49 FDA approved biologics, 15 (31%) were identified as producing nonclinical "infusion reactions". Of these 15 approved products, 12 were delayed ADA-mediated reactions (Mease, 2017). These include transformational products that have addressed significant unmet needs across multiple indications and patient groups. Among them were Obinutuzumab and Ofatumumab, anti-CD20 antibodies, Ipilimumab, a CTLA4-Ig fusion protein, Panitumumab, an anti-EGFR antibody, Natalizumab, an anti-alpha4-integrin antibody, Rilonacept, an anti-IL1β antibody, and Abatacept, an anti-CD80 antibody (Mease, 2017).