Subcutaneous Administration of Humanized, Fc-Engineered, Anti-CD20 Antibody: AME-133 Demonstrated Significant Dose Dependent B-Cell Depletion in Cynomolgus Monkeys (*Macaca fascicularis*)

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ABSTRACT

Background:
AME-133 is an anti-CD20 antibody and is Fc-engineered to be more potent than rituximab. Relative to rituximab, AME-133 has a 10-20 fold higher binding affinity for the CD20 epitope and approximately a 6-fold greater potency in ADCC assays. AME-133 is humanized to decrease immunogenicity which diminishes CDC activity and potentially reduces side effects. In phase I/II clinical studies of AME-133, relapsed/refractory patients with low affinity FcγIIIRa follicular non-Hodgkin's lymphoma (NHL) have shown excellent safety profiles and response rates (RR) greater than 30%.

Objective:
As a prelude to investigating the clinical efficacy and safety of AME-133 in subcutaneous (SC) formulation in human beings, we investigated the pharmacokinetics (PK) , B-cell depletion, and immunogenicity of AME-133 in cynomolgus monkeys at 3 different dose levels by SC route.

Materials and Methods:
A total of 48 (24 male and 24 female) cynomolgus monkeys (*Macaca fascicularis*) were randomly assigned to four dose groups of AME-133 administered SC route once weekly (0.0 mg/kg (vehicle), 0.6 mg/kg, 1.9 mg/kg and 6.0 mg/kg). Within the dose groups, there were two (6 week and 14 week) dosing schedules. In the 6 week dosing schedule, two-third of animals were sacrificed immediately after 6 weeks, and one-third of animals were sacrificed after 4 to 8 weeks of recovery period. In the 14 week dosing schedule, two-third of animals were sacrificed immediately after 14 weeks, and one-third of animals were sacrificed after 4 to 8 weeks of recovery period.

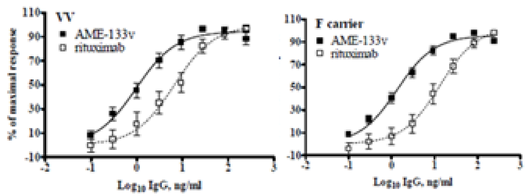
Results:
All dose levels of AME-133 substantially depleted B-cells in peripheral blood. The extent and duration of B-cell depletion was dose-dependent. The mid and high doses (1.9 mg/kg and 6.0 mg/kg) resulted in delayed return to normal during recovery in monkeys in 14 week dosing schedule.

Discussion and Conclusion:
Therapeutic concentrations of AME-133 may be achieved when administered by SC route. Sustained B-cell depletion throughout the dosing regimen of AME-133 is suggestive of less frequent dosing requirements. Administration by of AME-133 by SC route is simple, convenient, cost effective and could be administrated by patients themselves.

BACKGROUND AND OBJECTIVE

Background:
AME-133 is a monoclonal anti-CD20 antibody which has been engineered to be more potent than rituximab. It has a 10-20 fold higher binding affinity for the CD20 epitope and approximately 6-fold greater potency in ADCC assays relative to rituximab *(Figure 1)*.

Figure 1: Comparison of AME-133 and rituximab in ADCC Assays in both (A) favorable and (B) unfavorable genotypes of FcγIIIRa



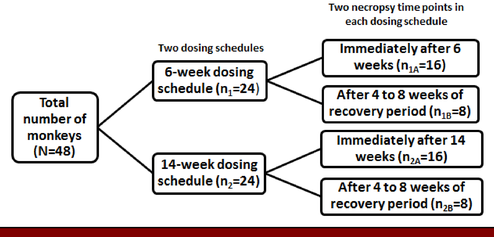
As a prelude to investigating the clinical efficacy of AME-133 in SC formulation, PK, B-cell depletion and immunogenicity study was conducted in cynomolgus monkeys.

The higher potency of AME-133 may provide benefit to patients with low affinity FcγIIIRa and enable SC administration (improved patient convenience). AME-133 is potentially safer than rituximab because it is humanized to decrease immunogenicity which diminishes CDC activity and potentially reduces side effects associated with tumor lysis syndrome.

MATERIALS AND METHODS

Study Design:

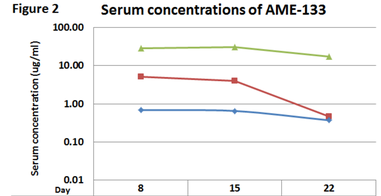
- Number of cynomolgus monkeys: 48 (24 male and 24 female).
- Route of administration: Subcutaneous (SC).
- Four dose cohorts of AME-133: Vehicle (n=12), 0.6 mg/kg (n=12), 1.9 mg/kg (n=12), 6.0 mg/kg (n=12)
- Two dosing schedules
  - Weekly dosing for 6 weeks
  - Weekly dosing for 14 weeks



RESULTS

Pharmacokinetics:
Circulating levels of AME-133 determined on day eight were within the expected range based on predicted values and exhibited dose-dependent relationship. Following the second injection of AME-133, a large proportion of the cynomolgus monkeys in all groups failed to demonstrate accumulation of antibody. By the third injection, all but one cynomolgus monkey (in the high-dose group) exhibited lower circulating levels of antibody compared to the previous trough (*Figure 2*). The lack of accumulation suggested the development of primate anti-human antibody (PAHA) response.

Figure 2 Serum concentrations of AME-133

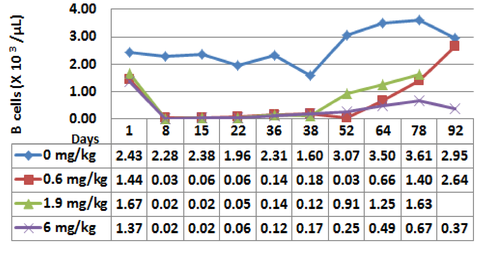


Day	8	15	22
6mg/kg s.c.	28.2177	31.2840	17.5823
1.9mg/kg s.c.	5.0970	4.0250	0.4673
0.6mg/kg s.c.	0.6828	0.6484	0.3693

B-cell Depletion:
AME-133 significantly decreased the B-lymphocyte population of the treated cynomolgus monkeys. Both males and females were influenced in a dose dependent manner. All dose levels of AME-133 substantially depleted the B-cells in the peripheral blood samples beginning with the first post-dose time point.

In the 6-week schedule group, B-cell count consistently remained low (0.02 – 0.88 X 10^3/µL) throughout the dosing regimen in all three AME-133 cohorts . The recovery of B-cell count was delayed in 6.0 mg/kg dosing cohort (*Figure 3A*).

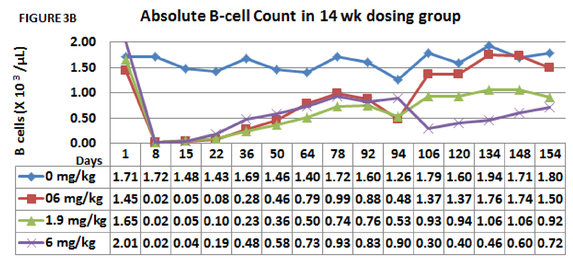
FIGURE 3A **Absolute B-cell Count in 6 wk dosing group**



Days	1	8	15	22	36	38	52	64	78	92
0 mg/kg	2.43	2.28	2.38	1.96	2.31	1.60	3.07	3.50	3.61	2.95
0.6 mg/kg	1.44	0.03	0.06	0.06	0.14	0.18	0.03	0.66	1.40	2.64
1.9 mg/kg	1.67	0.02	0.02	0.05	0.14	0.12	0.91	1.25	1.63	
6 mg/kg	1.37	0.02	0.02	0.06	0.12	0.17	0.25	0.49	0.67	0.37

In the 14-week schedule group, B-cell count consistently remained lower (0.02 – 0.14 X 10^3/µL) throughout the dosing regimen in all three AME-133 cohorts. The recovery of B-cell count was delayed in 1.9 mg/kg and 6.0 mg/kg dosing cohorts (*Figure 3B*).

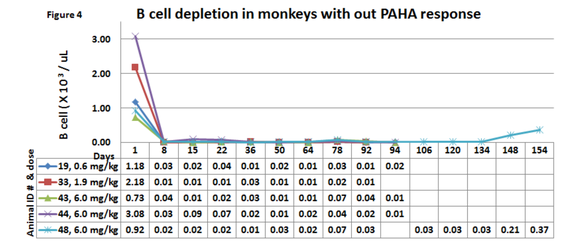
FIGURE 3B **Absolute B-cell Count in 14 wk dosing group**



Days	1	8	15	22	36	50	64	78	92	94	106	120	134	148	154
0 mg/kg	1.71	1.72	1.48	1.43	1.69	1.46	1.40	1.72	1.60	1.26	1.79	1.60	1.94	1.71	1.80
06 mg/kg	1.45	0.02	0.05	0.08	0.28	0.46	0.79	0.99	0.88	0.48	1.37	1.37	1.76	1.74	1.50
1.9 mg/kg	1.65	0.02	0.05	0.10	0.23	0.36	0.50	0.74	0.76	0.53	0.93	0.94	1.06	1.06	0.92
6 mg/kg	2.01	0.02	0.04	0.19	0.48	0.58	0.73	0.93	0.83	0.90	0.30	0.40	0.46	0.60	0.72

A total of 5 cynomolgus monkeys (one at 0.6 mg/kg, one at 1.9 mg/kg, and three at 0.6 mg/kg dose groups) did not show positive titer in PAHA assay, thus demonstrated a sustained B-cell depletion (*Figure 4*).

Figure 4 **B cell depletion in monkeys with out PAHA response**



Animal ID # & dose / Days	1	8	15	22	36	50	64	78	92	94	106	120	134	148	154
19, 0.6 mg/kg	1.18	0.03	0.02	0.04	0.01	0.02	0.01	0.03	0.01	0.02					
33, 1.9 mg/kg	2.18	0.01	0.01	0.01	0.03	0.01	0.01	0.02	0.01						
43, 6.0 mg/kg	0.73	0.04	0.01	0.02	0.03	0.01	0.01	0.07	0.04	0.01					
44, 6.0 mg/kg	3.08	0.03	0.09	0.07	0.02	0.01	0.02	0.04	0.02	0.01					
48, 6.0 mg/kg	0.92	0.02	0.02	0.02	0.01	0.03	0.02	0.07	0.03		0.03	0.03	0.03	0.21	0.37

DISCUSSION AND CONCLUSIONS

- AME-133 was well absorbed and caused rapid B-cell depletion at doses as low as 0.6 mg/kg. Therefore, therapeutic concentrations of AME-133 may be achieved when administered by SC route.
- A sustained B-cell depletion throughout the dosing regimen (in all three doses) and delayed return to baseline during recovery in 6-week dosing group (6 mg/kg cohort) and 14-week dosing group (1.9 mg/kg and 6.00 mg/kg cohorts) is suggestive of less frequent dosing requirements.
- These results justify further investigation of AME-133 administered by SC route in follicular lymphoma and other B-cell malignancies.

Disclosure: There are no relevant conflicts of interest to disclose

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