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Toxicity Profile of Interleukin 12 Attached to a Fully Human Albumin Binding Domain (F_HAB™) in Cynomolgus Macaques

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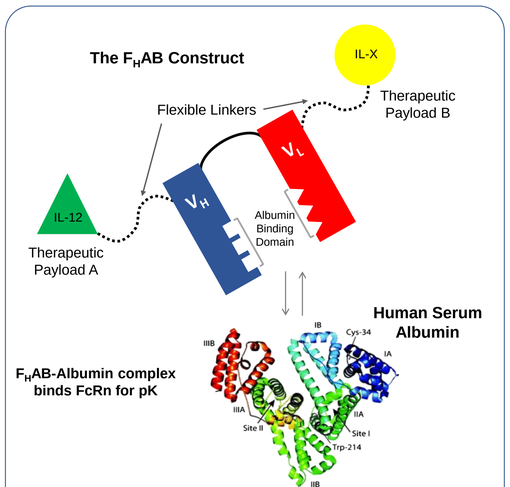
INTRODUCTION

Interleukin 12 (IL-12) is a well characterized immunomodulatory cytokine with potent activity against multiple tumor types. Early efforts to advance recombinant human IL-12 (rhIL-12) as a therapeutic were met with limited success in the clinic due to its short circulating half-life, leading developers to experiment with dosing regimens that often resulted in unacceptable safety outcomes. Sonnet's proprietary F_HAB technology for enhancing the activity of immunomodulators, such as IL-12, utilizes a fully human serum albumin-binding scFv domain. The F_HAB construct targets tumor tissue by binding GP60 and SPARC, provides a dose sparing effect for mitigating toxicity, and broadens the therapeutic window, resulting in improved pharmacokinetics (see Figure 2). In a B16F10 mouse melanoma model, compared to rIL-12, mouse IL12-F_HAB displayed 10-to-30-fold greater tumor inhibition at a common dose level. Similar *in vivo* efficacy was also observed with other cytokines: IL-15, GM-CSF and IL-18, and bispecific combinations.

Sonnet's Fully Human Albumin Binding (F_HAB) technology utilizes a single chain antibody fragment (scFv) capable of delivering one or two active drug compounds

- Therapeutic payloads attached via **flexible linker peptides**
- Following administration, F_HAB-derived **candidates bind to and "hitch-hike" on endogenous human serum albumin** for transport to target tissues
- F_HAB is designed to **bind, unbind and rebind to albumin** in an on-and-off fashion through a physical bonding mechanism, obviating the need for chemical conjugation

CLICK HERE TO VIEW A MUTLIMEDIA OVERVIEW OF THE F_HAB TECHNOLOGY



BACKGROUND & OBJECTIVES

- Previously generated data assessing the *in vitro* species compatibility showed that among the various animal models examined, the non-human primate was the only animal model relevant for toxicity and safety related endpoints of IL12-F_HAB
- Prior studies using the murine based construct of IL12-F_HAB displayed tolerance and pharmacokinetics consistent with the targeted therapeutic window, however this study marks the first *in vivo* assessment of fully human IL12-F_HAB
- The objectives of this study were:
  - Conduct a dose escalation to determine the Maximum Tolerated Dose of IL12-F_HAB in healthy cynomolgus macaques.
  - Assess the Pharmacokinetics of IL12-F_HAB following a single intravenous or subcutaneous injection
  - Determine the effects of IL12-F_HAB on clinical chemistry, hematology, and cytokines
  - Determine the effects of IL12-F_HAB on Immune cell proliferation and phenotype
  - Produce data to inform subsequent Repeat Dose Toxicity studies

STUDY DESIGN

Group No.	Dose Route	Clinical Dose Equivalent Fold	No. of Males	No. of Females
1	IV	282	1	1
2	SC	282	1	1
3	IV	141	1	1
4	SC	141	1	1
5	IV	70	1	1
6	SC	70	1	1

	Time Post Dose								
Item	**0h**	**1 h**	**4 h**	**24 h**	**48 h**	**96 h**	**144 h**	**312 h**	**480 h**
Bioanalysis / PK	-	X	X	X	X	X	X	X	X
Cytokine Panel	X			X	X	X			

	Time Post Dose				
Item	**Predose**	**Day 3**	**Day 7**	**Day 14**	**Day 21**
Clinical Chemistry	X	X	X	X	X
Hematology	X	X	X	X	X
Immunophenotyping	X	X	X	X	X

MATERIALS & METHODS

TEST SYSTEM

- All procedures and protocols were reviewed and approved by the Testing Facility's Institutional Animal Care and Use Committee.
- Mauritius cynomolgus monkeys were chosen as the appropriate animal model for this study as no non-animal models exist to characterize the effects required to meet the study objectives
  - All animals were 2.0 to 2.9 years of age, weighing between 2.3 and 3.3 kg at initial dosing; a 14-day acclimation period was conducted prior to initiation of study procedures.
  - Housing, Enrichment, and Veterinary Care was as specified in the USDA Animal Welfare Act (9 CFR, Parts 1, 2, and 3) and as described in the *Guide for the Care and Use of Laboratory Animals.*

IN-LIFE ASSESSMENTS

Parameter	Frequency (Minimum Required)	Comments
Mortality	At least twice daily (morning and afternoon) beginning upon transfer	Animals were observed within their cage unless necessary for identification or confirmation of possible findings.
Cageside Observations	At least once daily; from at least Week -1 Except on days where detailed clinical observations are scheduled.	Animals were observed within their cage unless necessary for identification or confirmation of possible findings.
Detailed Clinical Observations	Prior to dose and weekly thereafter	Animals were removed from the cage.
Individual Body Weights	Once pretest and then twice weekly	
Appetite Evaluation	Once daily; from at least Week -1 and throughout the study	Qualitatively measured

BIOANALYSIS / PHARMACOKINETICS

- Bioanalysis of IL12-F_HAB was conducted via two separate qualified ELISA methods which included a High Sensitivity and Low Sensitivity assay
- Pharmacokinetic characteristics were modeled via noncompartmental analysis using Phoenix WinNonlin Software

CLINICAL CHEMISTRY/ HEMATOLOGY/ CYTOKINES

- Comprehensive clinical chemistry and hematology panels were assessed using qualified and validated methods by the Testing Facility
- The samples were analyzed at the Testing Facility for IFN-γ, TNF-α, IL-6, IL-8, IL-10, IL-12 p40 and IL-1-beta (IL-12 p40 was run as a single plex). Analysis for the multi-plex and single-plex cytokines was conducted by a Luminex method. Samples were analyzed in duplicate.

IMMUNOPHENOTYPING

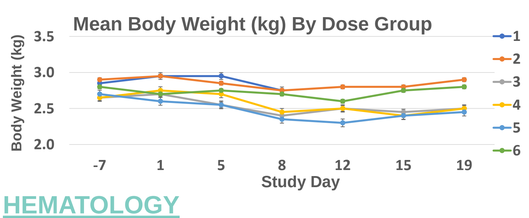
Antigen Marker(s)	Cell Population Identified
CD45+/CD14-/CD20+	B-lymphocytes
CD45+/CD14-/CD20-/CD159a-/CD3+	Total T-lymphocytes
CD45+/CD14-/CD20-/CD159a-/CD3+/CD4+/CD8-	T-helper lymphocytes
CD45+/CD14-/CD20-/CD159a-/CD3+/CD4-/CD8+	T-cytotoxic lymphocytes
CD45+/CD14-/CD20-/CD3-/CD159a+	Natural-killer cells

RESULTS

CLINICAL OBSERVATIONS

- IL12-F_HAB-related abnormal clinical observations were observed in both males and females during the dosing period and included hunched postured, mild/moderate dehydration, decreased activity, soft/liquid feces, mild, intermittent tremors and reduced appetite.
- All abnormal clinical signs resolved by approximately 15 days post dose.
- Animals in Group 1 were euthanized on Study Day 10 to assess pathology which included spleen and lymph node enlargement as well as adrenal gland enlargement and atrophy of the thymus in one animal.

BODY WEIGHT



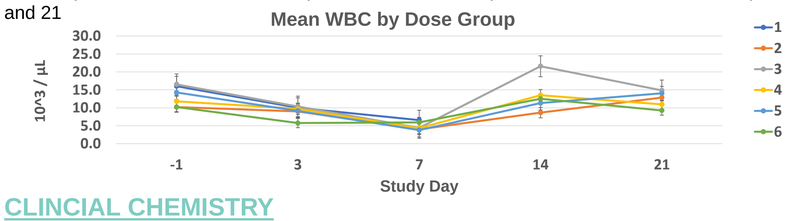
- **Minor body weight loss observed in most animals compared to Day -7**
- **No bodyweight loss exceeded 15% of beginning weight**
- **Bodyweight rebounded by Day 19 to comparable Day -7 levels**

HEMATOLOGY

Hematology changes related to IL12-F_HAB included overall decrease in all groups in white blood cells (WBC), neutrophils, lymphocytes and monocytes, eosinophils and basophils. The decrease was observed on Day 3 and 7, followed by the recovery and levels comparable to pretest values on Day 14 and/or 21. Mean platelet (PLT) values were decreased on Day 3, 7 and 14, and returned to pretest values on Day 21. A decrease in red blood cell (RBC) compared to Day -1 values, was observed for animals on Day 7 and throughout the study.

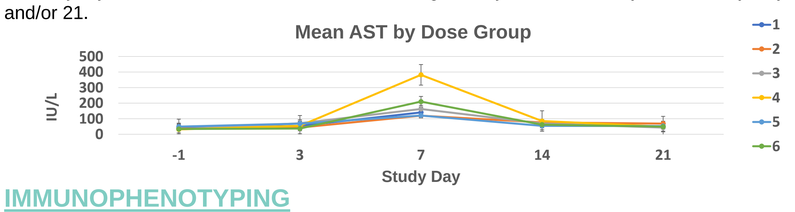
HEMATOLOGY

The effect on RBCs correlated with decreased hemoglobin and hematocrit and increased Red Blood Cell Distribution Width on Days 14 and 21 indicating depletion of the RBCs. The reticulocyte count decreased on Day 3 and 7, followed by an increase in values on Days 14 and 21



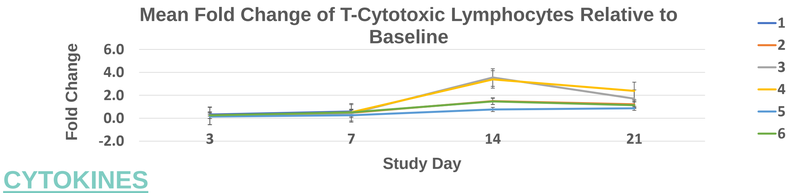
CLINICIAL CHEMISTRY

The clinical chemistry changes related to IL12-F_HAB administration included mildly increased values for aspartate aminotransferase (AST) on Day 7, and total bilirubin (TBIL) on Day 3. In a majority of animals, the level of AST and TBIL gradually returned to Day -1 values by Day 14 and/or 21.



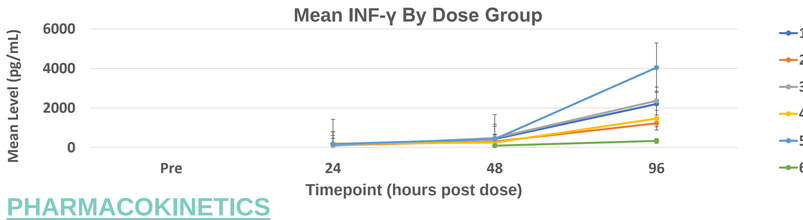
IMMUNOPHENOTYPING

The single dose of IL12-F_HAB resulted in the margination of peripheral lymphocytes causing a decrease of peripheral absolute total T, T-helper, T-cytotoxic, and Natural-killer cells for all animals by Day 3. Group 2, cell populations of T-lymphocytes, T-helper lymphocytes, T- cytotoxic lymphocytes recovered back to baseline by Day 21. For T-Lymphocytes, Group 4 and Group 6 animals all recovered back to baseline values by Day 21.



CYTOKINES

Predose cytokine values were < LLOQ except for TNF-α in a single Group 1 animal. There were no detectable responses for IL-10, IL-1β, or TNF-α. IL-6 was detected for a single animal in Group 4 and 5 at 96h post dose. There was an IFN-γ response for all animals except a single animal in Group 6; responses increased over time with the highest IFN-γ concentrations reported at the 96h time point for each animal with a response.



PHARMACOKINETICS

Dose Group	Item	C_{max} (ng/ml)	T_{max} (h)	AUC_{last} (h*ng/ml)	AUC_{∞} (h*ng/ml)	Vz_F (ml/kg)	Cl_F (ml/h/kg)	Vz (ml/kg)	Cl (ml/h/kg)	$T_{1/2}$ (h)
Group 1	N	2	2	2	2			2	2	2
	Mean	9486	1	272392	273246			35	1	26
	CV%	25	0	7	7			4	7	3
Group 2	N	2	2	2	2	2	2			2
	Mean	1184	24	124197	124218	106	2			36
	CV%	30	0	8	8	13	8			5
Group 3	N	2	2	2	2			2	2	2
	Mean	3072	1	109562	109580			51	1	30
	CV%	10	0	34	34			20	34	14
Group 4	N	2	2	2	2	2	2			2
	Mean	560	24	52085	52126	123	2			36
	CV%	3	0	16	16	1	16			17
Group 5	N	2	2	2	2			2	2	2
	Mean	1283	1	44941	44954			53	1	26
	CV%	10	0	17	17			20	17	3
Group 6	N	2	2	2	2	2	2			2
	Mean	348	24	22108	23392	205	3			48
	CV%	73	0	52	41	48	41			7

CONCLUSIONS

- **A single subcutaneous administration of IL12-F_HAB was generally tolerated in cynomolgus monkeys at 282-fold of the human clinical dose equivalent.**
- **A single intravenous administration of IL12-F_HAB was tolerated up to 141-fold of the human clinical dose equivalent.**
- **IL12-F_HAB-related changes in clinical observations, body weight loss, clinical pathology, cytokines and immunophenotyping occurred.**
- **Most parameters recovered to pre-study values by the end of the 3-week observation period.**
- **Repeat Dose Toxicity studies are needed to mirror the anticipated clinical dosing regimen.**