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The enhanced immunopharmacology of VIB4920, a novel Tn3 fusion protein and CD40L antagonist, and assessment of its safety profile in cynomolgus monkeys

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SUPPORTING INFORMATION

Methods

Anti-drug-antibody (ADA) evaluation

Sample collection

In all studies, blood samples of approximately 1 mL were collected from all animals via the femoral vein for anti-VIB4920 antibody analysis and processed to platelet poor plasma.

In Study 1, samples were collected pre-test, on Days 15 and 29, and every other week during the recovery period. In Study 2, samples were collected pre-test (Day -28), pre-dose on Days 15 and 29, and on Days 64, 92, 120, 148, 176, and 190 during the recovery period and prior to the recovery necropsy (Day 224). In Study 3, samples were collected once pre-study, and pre-dose on Days 15, 29, 64, 120, and 190 during the dosing phase, and once on Day 260 during the recovery period.

Analysis

The detection of ADA to VIB4920 in the cynomolgus monkey K_2EDTA platelet poor-plasma samples (CMP) was determined using a qualified (Study 1) or validated (Studies 2 and 3) electrochemiluminescence (ECL) analytical test. The method is a bridging immunoassay format in which a bare MesoScale Discovery (MSD) high-bind plate was coated with VIB4920 and incubated overnight at 2–8°C. Following a plate block step, quality controls (QCs) and samples diluted to the minimum required dilution of 1:30 in a buffer containing anti-CD40L antibody blocking antibody were transferred to the blocked plate and incubated at room temperature for 1 hour. Two 1 hour incubation steps with biotin-labeled VIB4920 and streptavidin Sulfo-TAG (Study 1) or a single 1 hour incubation with Sulfo-TAG-labeled VIB4920 (Studies 2 and 3) completed the bridging immunocomplex. MSD Read Buffer T was added to detect the binding complex and signal, in the form of ECL (light) units, was

generated by an MSD Sector™ Imager Model 6000 instrument. The resulting ECL signal was proportional to the level of ADA present in the sample.

Specialised clinical pathology

D-Dimer analysis

For all studies, blood samples (approximately 1.2 mL) were collected from all animals via the femoral vein, placed into tubes containing sodium citrate as an anticoagulant, and stored at room temperature until analysis, which occurred within 6 hours of collection. The plasma D-Dimer analysis method uses anti-D-Dimer antibodies. The anti-D-Dimer antibodies react with plasma samples containing D-Dimer to form antigen/antibody complexes which, following agglutination, are measured turbidimetrically. The method was calibrated using a six-point standard curve.

In Studies 1 and 2, samples were collected pre-test and on Days 1 and 22 at 12, 24, 36, and 48 hours post-dose. In Study 3, blood samples were collected once pre-study and on Days 1, 29, 57, 85, 113, 141, and 169 at 12 hour and 48 hours post-dose, once prior to terminal necropsy, and on Days 372, 374, 386, and 388 for recovery animals.

Analyses of thrombin-anti-thrombin (TAT) complexes

For all studies, blood samples (approximately 1.2 mL) were collected from all animals via the femoral vein, placed into sodium citrate tubes, and stored at room temperature until centrifuged to collect plasma. An immunoassay kit developed by an Enzygnost® TAT was used to measure the concentration of TAT complex in plasma.

A pre-blocked mictrotitre plate coated with an antibody against thrombin was provided with the kit. Prepared standards, controls, and samples containing TAT were added to the appropriate wells. After incubation, unbound material was washed away and a peroxidase-conjugated antibody to human antithrombin III was added. Following incubation and plate washing, substrate was added and colour developed by the enzymatic reaction of the peroxidase-conjugate on the substrate, which was directly proportional to the amount of

TAT complex present in the sample. The color development was stopped with the addition of an acidic solution and the intensity of the color was measured at 490 nm.

In Study 1, samples were collected pre-test and on Days 1 and 22 at 10 minutes, and 1 and 3 hours post-dose. In Study 2, samples were collected pre-test, pre-dose and at 10 minutes and 1 and 3 hours post-dose on Days 1 and 22. In Study 3, samples were collected pre-study, and on Days 1 and 190 at 10 minutes, and 1 and 3 hours post-dose, and on Days 29, 57, 85, 113, 141 and 169 at 1 and 3 hours post-dose.

Platelet function screening (PFA-100), platelet collagen/epinephrine closure time analysis

For all studies, blood samples (approximately 1.8 mL) were collected from the femoral vein, using a 21-gauge needle, directly into evacuated plastic tubes containing buffered 3.2% (0.109 M) sodium citrate. The tubes were gently inverted 1–2 times and stored at room temperature until analysis.

In Studies 1 and 2, samples were collected pre-test and on Days 1 and 22 at 10 minutes and 1 and 3 hours post-dose. In Study 3, samples were collected from all animals once pre-study, on Days 1 and 190 at 10 minutes, 1 hour and 3 hours post-dose, and on Days 29, 57, 85, 113, 141, and 169 at 1 and 3 hours post-dose.

Soluble CD40L analysis

The concentration of total sCD40L in cynomolgus monkey platelet poor plasma samples was determined using a qualified sandwich ELISA method (Qualification Report BAS4920-0006-CynoPlasma-TotalPD-Qual) as described below and in Bioanalytical Procedure, “Assay for the Measurement of Total Soluble CD40L Ligand in Cynomolgus Monkey Plasma.” The method procedure used components provided in a commercially available kit, including a precoated microtiter plate, sample diluent, detection antibody, and phosphoric acid stop solution. Calibration standards were prepared in kit-supplied sample diluent and the QCs were prepared in cynomolgus monkey platelet poor plasma. All standards, QCs, and samples were then diluted to the minimum required dilution (MRD) of 1:5 with sample diluent containing 3.75 µg/mL MEDI4920 prior to being applied to the precoated assay plate. Calibrators, QCs, and samples were incubated for 1.5 hours at room temperature (RT) while

shaking to allow binding of sCD40L to the immobilized anti-sCD40L monoclonal antibody. After washing away unbound material, horseradish peroxidase-conjugated anti-sCD40L monoclonal antibody was added to the wells and incubated for 1 hour at RT while shaking. The addition of tetramethylbenzidine substrate solution resulted in color development that was directly proportional to the level of analyte in the sample. Once stopped with 1 M phosphoric acid, the plate was read on a spectrophotometer at 450 nm, and data analyzed in SoftMax® Pro (SMP) software, version 5.4. The concentration of sCD40L in cynomolgus monkey platelet poor plasma samples was determined relative to the standard curve, which was fit to a 5-parameter logistic fit with 1/y2 weighting. The quantitative range was 390.63 to 25000.00 pg/mL total sCD40L.

Results

Anti-drug-antibody (ADA) evaluation

In Study 1, 2/6 (33%) animals in the control group and 12/24 (50%) VIB4920-treated animals tested positive for ADA. The presence of ADAs did not noticeably affect the plasma concentration or clearance of VIB4920.

In Study 2, 3/12 (25%) animals in the control group and 7/24 (29%) VIB4920-treated animals tested positive for ADA. Positive ADA results did not affect VIB4920 toxicokinetics.

In Study 3, 6/12 (50%) animals in the control group, and 2/12 (17%), 0/12, 1/12 (8%), and 0/12 animals treated with SC 125 mg/kg SC 250 mg/kg, IV 150 mg/kg and IV 300 mg/kg, respectively, tested positive for ADA. The presence of ADA did not impact on VIB4920 exposure.
The percentage of pre-dose ADA positive animals suggests that there may be some false positive results due to sCD40L bridging the drug complex.

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In Study 1, no VIB4920-related effects on D-Dimers or TAT complex concentrations were observed at any dose level at any interval. All fluctuations in mean and individual values were considered within an acceptable range for biologic variation. No test article-related

effects on PFA-100 closure times were observed at any dose level at any interval. Although individual animal closure times in all groups (including controls) tended to be mildly prolonged at pre-test relative to historical baselines. Most individual animal values remained within expected ranges throughout the study in all treatment groups at all intervals.

In Study 2, there were no VIB4920-related effects on coagulation times, D-dimers or TAT complex generation in either sex at any dose level. There were no test article-related effects on PFA-100 closure times in either sex at any dose level. All individual values were considered within an acceptable range for biological and analytical variation.

In Study 3, there were no VIB4920-related changes in TAT complexes. Intergroup differences in TAT complexes were not considered VIB4920 related and were considered attributed to biologic variation because they were sporadic and/or similar to fluctuations in control values. VIB4920-related, non-adverse increased D-dimer concentrations were observed in some animals treated with IV 150 or 300 mg/kg VIB4920. The D-dimer concentration was higher than the highest individual control value (4.74 μg/mL for males and 2.71 μg/mL for females) at one or more time points for several animals in the IV 300 mg/kg and SC 250 mg/kg VIB4920 treatment groups. There were no VIB4920-related changes in PFA-100 closure time.

sCD40L

Within each study, total sCD40L (free sCD40L + sCD40L bound to VIB4920) was determined as a measure of target engagement. Baseline levels of sCD40L ranged from undetectable to maximum values of 21, 99, and 25 ng/mL in Study 1, 2, and 3, respectively, with wide variability between animals. VIB4920 does bind to sCD40L through the mutated Tn3 portion of the molecule; wild-type Tn3 does not bind sCD40L. Over time and dose-dependently, the concentration of total sCD40L increased, indicating binding of VIB4290 to sCD40L and hence, peripheral target engagement. Higher levels of sCD40L don’t appear to affect the PK parameters of VIB4920 or impact the VIB4920 mechanism of action.

SUPPORTING INFORMATION – TABLES

Table 1. Study designs and dosing schedules

Study	Study length		Test article	Administration route	VIB4920 concentration (mg/kg)	Number of animals (males/females)	
	Treatment period (months)	Recovery period (weeks)				Treatment period	Recovery period
1[a]	1	20	Vehicle	IV bolus/SC	0	3/0	3/0
	1	20	VIB4920	IV bolus	5	3/0	3/0
	1	20	VIB4920	IV bolus	50	3/0	3/0
	1	20	VIB4920	IV bolus	150	3/0	3/0
	1	20	VIB4920	SC	150	3/0	3/0
2[a]	1	27	Vehicle	IV infusion	0	3/3	3/3
	1	27	VIB4920	IV infusion	50	3/3	0/0
	1	27	VIB4920	IV infusion	150	3/3	0/0
	1	27	VIB4920	IV infusion	300	3/3	3/3
3[b]	7	29	Vehicle	IV infusion/SC	0	3/3	3/3
	7	29	VIB4920	SC	125	3/3	3/3
	7	29	VIB4920	SC	250	3/3	3/3
	7	29	VIB4920	IV infusion	150	3/3	3/3
	7	29	VIB4920	IV infusion	300	3/3	3/3

[a]The vehicle and VIB4920 were administered on Days 1, 8, 15, 22 and 29.

[b]The vehicle and VIB4920 were administered weekly on Days 1–190 (for a total of 28 doses).

IV, intravenous; SC, subcutaneous.

Table 2. Pharmacokinetic and pharmacodynamic analysis sample collection schedule by study

	Study 1			Study 2				Study 3			
Study day	**VIB4920 administration**	**PK sample collected**	**PD sample collected**	**Group**	**VIB4920 administration**	**PK sample collected**	**PD sample collected**	**Group**	**VIB4920 administration**	**PK sample collected**	**PD sample collected**
-28			X[b,c]	1 to 4			X[b,c]	1 to 5			X[b,c]
-22			X[b,c]	1 to 4			X[b,c]	1 to 5			X[b,c]
-19			X[b,c]					1 to 5			
-18				1 to 4			X[b,c]	1 to 5			X[b,c]
-15			X[b,c]	1 to 4			X[b,c]	1 to 5			X[b,c]
-8			X[b,c]	1 to 4			X[b,c]	1 to 5			X[b,c]
-1			X[b,c]	1 to 4			X[b,c]	1 to 5			X[b,c]
1[a]	X	X		1 to 4	X	X		1 to 5	X	X	
2		X		1 to 4		X		1 to 5		X	
3		X						1 to 5			X[b,c]
4		X		1 to 4		X		1 to 5		X	
5				1 to 4		X					
7								1 to 5			X[b,c]
8[a]		X	X[b]	1 to 4	X	X	X[b,d]	1 to 5	X	X	X[d]
10								1 to 5			X[b,c]
11				1 to 4		X	X[b]	1 to 5		X	
14								1 to 5			X[b,c]
15[a]	X	X	X[b]	1 to 4	X	X	X[b,d]	1 to 5	X	X	
18			X[b]	1 to 4		X	X[b]	1 to 5		X	
21								1 to 5			X[b,c]

22[a]	X	X	X[b]	1 to 4	X	X	X[b,d]	1 to 5	X	X	
27			X[b]								
28				1 to 4		X	X[b]	1 to 5			X[b,c]
29[a,e]	X	X	X[b]	1 to 4	X	X		1 to 5	X	X	
30				1 to 4		X					
31											
32		X		1 to 4		X					
33				1,4		X					
35								1 to 5			X[b,c]
36		X	X[b]	1,4		X	X[b]				
42								1 to 5			X[b,c]
43				1,4		X	X[b]				
50		X		1,4		X					
64		X		1,4		X		1 to 5	X	X	
78				1,4		X					
92				1,4		X					
105		X									
106				1,4		X					
120		X		1,4		X		1 to 5	X	X	
134		X		1,4		X					
142			X[c]								
145			X[c]								
148		X		1,4		X					
149			X[c]								
156			X[c]								

162		X	X[c]	1,4		X					
170											
176				1,4		X					
182				1,4			X[c]				
185				1,4			X[c]				
189				1,4			X[c]				
190[a]				1,4		X		1 to 5	X	X	
191								1 to 5		X	
192				1,4			X[c]				
193								1 to 5[f]		X	
196				1,4			X[c]				
197				1,4		X		1 to 5[f]		X	
203				1,4			X[c]				
204				1,4		X					
210				1,4			X[c]				
217				1,4			X[c]				
224				1,4		X					
260								1 to 5[f]		X	
316								1 to 5[f]		X	

[a]In Study 1, three samples were collected on Days 1 and 29: at pre-dose and 30 min and 12 hours after VIB4920 administration.

In Study 2, samples were collected at pre-dose and at end of infusion (30 min after VIB4920 administration). At Days 1 and 29, samples were also collected 8 hours after VIB4920 administration.

In Study 3, at Days 1 and 190, PK samples were collected pre-dose (Groups 1 to 5), 2 and 12 h after VIB4920 SC administration (Groups 1, 2 and 3) and at end of infusion (30 min post-dose) and 8 hours after VIB4920 IV administration (Groups 4 and 5).

[b]Sample collected for KLH analysis.

[c]Sample collected for TT analysis.

[d]At Days 8, 15 and 22, samples for KLH analysis were collected at pre-dose only.

[e]At Day 29 (Study 3), PK samples were collected pre-dose (Groups 1 to 5), 2 hours after VIB4920 SC administration (Groups 1, 2 and 3) and at end of infusion (30 min after VIB4920 IV administration; Groups 4 and 5).

[f]Recovery animals.

Study 1: Group 1, vehicle; Group 2, IV 5 mg/kg VIB4920; Group 3, IV 50 mg/kg VIB4920; Group 4, IV 150 mg/kg VIB4920; Group 5, SC 150 mg/kg VIB4920.

Study 2: Group 1, vehicle; Group 2, IV 50 mg/kg VIB4920; Group 3, IV 150 mg/kg VIB4920; Group 4, IV 300 mg/kg VIB4920.

Study 3: Group 1, vehicle; Group 2, SC 125 mg/g VIB4920; Group 3, SC 250 mg/g VIB4920; Group 4, IV 150 mg/kg VIB4920; Group 5, IV 300 mg/kg VIB4920.

IV, intravenous; PD, pharmacodynamic; PK, pharmacokinetic; KLH, keyhole limpet haemocyanin; SC, subcutaneous; TT, tetanus toxoid.

Table 3. Antibodies used for determination of immune cell populations for Studies 1 and 2

Antibody	**Clone**	**Manufacturer and Cat#**
Anti-Non-Human Primate CD45-FITC	DO58-1283	BD Pharmingen, Cat# 557803
Anti-Human CD3-PE-Cy7	SP34-2	BD Pharmingen, Cat# 557749
Anti-Human CD4-PE	M-T477	BDPharmingen, Cat# 556616
Anti-Human CD8-PE-Cy5	B9.11	Beckman Coulter, Cat# IM2638U
Anti-Human CD159a-APC	Z199	Beckman Coulter, Cat# A60797
Anti-Human CD20-PE	2H7	BD Pharmingen, Cat# 556633
Anti-Human CD14-ECD	RMO52	Beckman Coulter, Cat# IM2707U

Table 4. Antibodies used for determination of immune cell populations for Study 3

Antibody	**Clone**	**Manufacturer**	**Catalog Number**
CD2-FITC	SFC13Pt2H9	Coulter	B36533
CD3-PE-Cy7	SP34-2	BD Pharmingen	557749
CD4-PE	L200	BD Pharmingen	550630
CD8-APC	B9.11	Coulter	IM2469U
CD14-FITC	M5E2	BD Pharmingen	555397
CD16-PE	3G8	BD Pharmingen	555407
CD20-ECD	B9E9	Coulter	IM3607U

Table 4. Tissue collected for histopathology

	Study 1		Study 2		Study 3	
Tissue	Collect	Weigh	Collect	Weigh	Collect	Weigh
Adrenal[a]	X	X	X	X	X	X
Aorta	X		X		X	
Axillary lymph node[b]	X		X			
Bone marrow smear (2 collected)[c]			X		X	
Bone with marrow (femur)[b]	X		X		X	
Bone with marrow (sternum)[b]	X		X		X	
Brain (cerebrum[c], midbrain, cerebellum, medulla/pons)	X	X	X	X	X	X
Epididymis[a]	X		X	X	X	X
Eye (including optic nerve and macula)[a,d]	X		X		X	
Femur					X	
Gallbladder	X		X		X	
Gut-associated lymphoid tissue	X		X		X	
Oesophagus	X		X		X	
Stomach (cardia, fundus and pylorus)	X		X		X	
Duodenum	X		X		X	
Jejunum	X		X		X	
Ileum	X		X		X	
Cecum	X		X		X	
Colon	X		X		X	
Rectum	X		X		X	
Testis[a,b,e]	X	X	X	X	X	X
Gross lesions	X		X		X	
Heart[b]	X	X	X	X	X	X
Identification site					X	
Inguinal lymph node	X		X			
Injection site(s): SC and/or IV[b]	X		X		X	

Kidney[a,b]	X	X	X	X	X	X
Lacrimal gland	X		X		X	
Liver (3 sections collected)[a,b]	X	X	X	X	X	X
Lung with bronchi (collected whole)[a,b]	X	X	X	X	X	X
Mandibular lymph node[c] (2 collected)	X		X		X	
Mammary gland (females only)			X		X	
Mesenteric lymph node[b]	X		X		X	
Ovary[a,b]			X	X	X	X
Oviduct[a]			X		X	
Pancreas[b]	X		X		X	
Pituitary	X	X	X	X	X	X
Prostate[b]	X	X	X	X	X	X
Salivary gland, mandibular (2 collected)[f]	X	X	X	X	X	
Sciatic nerve	X		X		X	
Seminal vesicle[a]	X		X		X	
Skeletal muscle, rectus fomoris[b]	X		X		X	
Skin[b]	X		X		X	
Spinal cord (cervical, thoracic, lumbar)	X		X		X	
Spleen[b]	X	X	X	X	X	X
Sternum					X	
Thymus[b]	X	X	X	X	X	X
Thyroid/parathyroid[a,b]	X	X	X	X	X	X
Tongue	X		X		X	
Trachea	X		X		X	
Tracheobronchial lymph node[b]	X		X			
Ureter					X	
Urinary bladder	X		X		X	
Uterus/Cervix (body, endometrium)[b]			X		X	X

Vagina			X		X	

[a]Paired organ.

[b]Snap frozen (if preserved).

[c]Bone marrow smears were collected at the scheduled necropsy and held. Smears were allowed to air dry and were not fixed in formalin.

[d]Preserved in Davidson's fixative.

[e]Preserved in modified Davidson's fixative (Study 3 only).

[f]Only the right mandibular salivary gland was weighed.

IV; intravenous; SC, subcutaneous.