

FIGURE 1

BMS-986325 Heavy Chain Amino Acid Sequence:

QVQLVQSGAEVKKPGSSVKVSCKASGYAFTSYWMHWVRQAPGQGLEWMGQINPTTGRSQYN
EKFKTRVTITADKSTSTAYMELSSLRSEDTAVYYCARWGLQPFAYWGQGLTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKPKSCDKTHTCPPCPAPELLGGKSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSV
MHEALHNHYTQKSLSLSPG

(SEQ ID NO: 5)

BMS-986325 Light Chain Amino Acid Sequence:

DIQMTQSPSFSLASVGDRTTITCKASQDVSTAWAYQQKPGKAPKLLIYSASYRYTGVPSR
FSGSGSGTDFTLTISSLQPEDFATYYCQQHYSTPWTFGGGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVCLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSLSSTLTLSK
ADYEEKHKVYACEVTHQGLSPVTKSFNRGEC

(SEQ ID NO: 11)

FIGURE 2A

Primary Pharmacodynamics		
Type of Study	Test System	Findings
CD40 binding affinity	Measurement of BMS-986325 binding to the extracellular domain of human, cynomolgus, rat, and mouse CD40 by SPR	BMS-986325 bound to human CD40 with a K _d of 5.3 nM and to cynomolgus CD40 with a K _d of 6.7 nM. Binding to mouse and rat CD40 was undetectable.
Binding to FcγRs	Measurement of BMS-986325 binding to the extracellular domain of CD32, CD16, and CD64 by SPR	Binding of BMS-986325 to CD16 and CD32 was undetectable. Binding of BMS-986325 was reduced 100× relative to wild type IgG1 containing proteins (K _d 17 nM).
Inhibition of B-cell proliferation	Soluble CD40L trimer stimulation of human tonsillar B cells	BMS-986325 inhibited B-cell proliferation with an IC ₅₀ of 0.04 ± 0.03 nM (5.6 ± 3.9 ng/ml; n=10 donors).
	CD40L-expressing CHO cell stimulation of tonsillar B-cell proliferation	BMS-986325 inhibited B-cell proliferation with an IC ₅₀ of 0.28 ± 0.07 nM (39.9 ± 9.8 ng/ml; n=8 donors).
Whole blood human B-cell activation	Soluble CD40L trimer stimulation of human B cells in whole blood	BMS-986325 inhibits CD86 upregulation with an IC ₅₀ of 0.24 ± 0.06 nM (34.2 ± 9.1 ng/ml; n=10 donors). BMS-986325 inhibits CD54 upregulation with an IC ₅₀ of 0.24 ± 0.06 nM (34.6 ± 8.7 ng/ml; n=10 donors).
Whole blood cynomolgus B-cell activation	Soluble CD40L trimer stimulation of cynomolgus B cells in whole blood	BMS-986325 inhibits CD86 upregulation with an IC ₅₀ of 0.25 ± 0.09 nM (36.4 ± 12.4 ng/ml; n=8 donors).
Inhibition of cynomolgus B-cell proliferation	Stimulation of cynomolgus splenic B cells with soluble CD40L trimer or CD40L-expressing CHO cells	BMS-986325 inhibits splenic B-cell proliferation driven by CD40L-trimer with an IC ₅₀ of 0.19 ± 0.18 nM (27.8 ± 25.9 ng/ml; n=6 donors) and that driven by CD40L-expressing CHO cells with an IC ₅₀ of 4.5 ± 5.4 nM (650.5 ± 782.7 ng/ml; n=6 donors).

FIGURE 2B

Primary Pharmacodynamics		
Type of Study	Test System	Findings
Inhibition of DC activation	Monocyte-derived DCs	BMS-986325 suppressed CHO CD40L-induced IL-6 production with an IC ₅₀ of 0.08 ± 0.04 nM (11.3 ± 5.3 µg/ml) and TNFα production with an IC ₅₀ of 0.1 ± 0.03 nM (13.3 ± 4.8 µg/ml); n=4 donors.
Binding of BMS-986325 to B cells	Direct binding of AF488-labeled BMS-986325 to B cells in human whole blood	AF488-labeled-BMS-986325 bound to CD20-positive B cells in human whole blood with a potency (EC ₅₀) of 0.90 ± 0.45 nM (130.4 ± 65.9 ng/ml; n=4 donors).
	Direct binding of AF488-labeled BMS-986325 to B cells in cynomolgus monkey whole blood	AF488-labeled-BMS-986325 bound to CD20-positive B cells in cynomolgus whole blood with a potency (EC ₅₀) of 1.0 nM (149.5 ng/ml; n=2 donors).
	Binding of BMS-986325 to B cells in cynomolgus whole blood using secondary anti-human IgG1-PE for detection	BMS-986325 bound to CD20-positive B cells in cynomolgus monkey whole blood with a potency (EC ₅₀) of 0.7 ± 0.15 nM (106.1 ± 22.6 ng/ml; n=4 donors).
Assay for agonism (B-cell proliferation or cytokine production)	BMS-986325 ability to stimulate tonsillar human B-cell proliferation or IL-6 production +/- IL-4	BMS-986325 (at concentrations up to 10 µg/ml) did not increase B-cell proliferation or IL-6 production either alone or in combination of IL-4 with tonsillar B cells from 10 donors.
Assay for agonism (iDC activation or cytokine production)	Immature DCs incubated with BMS-986325 either alone or with the addition of FcγR-expressing cells (CHO-CD32)	BMS-986325, either alone or in the presence of CD32 expressing CHO cells, did not increase activation markers (CD86 or CD54 cell surface expression) or IL-6 production in immature DCs. Experiments were performed with cells from 10 different donors and concentrations up to 100 µg/ml of BMS-986325.
Activation of human B cells in whole blood	Assay for the ability of BMS-986325 to increase CD54, CD69 or CD86 expression on B cells in human whole blood	BMS-986325 did not significantly increase CD54, CD69 or CD86 expression on B cells in whole blood from 6 donors at concentrations up to 12 µg/ml.

FIGURE 2C

Primary Pharmacodynamics		
Type of Study	Test System	Findings
Activation of CD40-NFκB reporter assay	Sensitive CD40-NFκB reporter assay in RAMOS B cell line +/- co-expression with FcγR (CD32)	BMS-986325 did not activate CD40-NFκB reporter at concentrations up to 10 μg/ml (n=3 independent experiments) either with or without CD32 co-expression.
Lack of effector function with BMS-986325	ADCC, CDC, and ADCP	BMS-986325 did not induce ADCC-, CDC- or ADCP-mediated killing of CD40-expressing target cells. BMS 986325 did not induce ADCC-mediated lysis by NK effector cells on Raji cells expressing CD40, whereas positive control rituximab did. BMS-986325 did not induce CDC, whereas positive control rituximab did. BMS-986325 did not induce phagocytosis of CD40-expressing Raji cells.

FIGURE 3

Type of Study/ Species/ Strain	Schedule/ Route/ Duration of Study/ Vehicle/ Formulation	Range of Doses (mg/kg)	Animals per group (M/F)	Noteworthy Findings
Primary Pharmacodynamics				
In vivo RO, target engagem ent and PD responses	RO and inhibition of soluble CD40L trimer stimulation of B-cell activation and T cell- dependent antibody responses to KLH immunization	1, 10, and 100 mg/kg SC	2M/2F	There was a dose-dependent duration of B-cell RO and PD responses in dosed animals. Complete RO was observed at all doses through Day 8, with RO falling to baseline by Day 18 for 1 mg/kg, and by Day 43 for 10 mg/kg. Full RO was maintained for the entire 43 days of the study at 100 mg/kg. On average, complete inhibition of a PD response (CD40L-induced B-cell activation) was observed through Day 8 at all doses. Recovery of the PD response was dose-dependent, observed by Day 15 at 1 mg/kg, Day 43 at 10 mg/kg, and maintenance of the PD response through Day 43 at 100 mg/kg. Additionally, suppression of the antibody response to an immunization with KLH was observed when ROs were maintained at ~ > 90%, through Day 29 at doses $\geq$ 10 mg/kg.

FIGURE 4

Projected Safety and Efficacy Margins of BMS-986325 for SAD Intravenous Doses (Part A1-A9)						
Cohort Process	Dose ^a (mg)	Predicted Exposure		NOAEL ^b Approach		MABEL Approach
		C _{max} ^a (µg/mL)	AUC(INF) ^a (µg/mL·h)	C _{max} Margin	AUC Margin	Max CD40 RO ^{a,c} (%)
A1	0.3	0.09	0.57	26,111	1,192,982	39.5
A2	1	0.3	2.4	7,833	283,333	35.9
A3	3	1	12	2,350	56,667	64.7
A4	10	4	79	588	8,608	86.6
A5	30	11	436	214	1560	95.2
A6	100	37	2,495	64	273	98.5
A7	300	111	11,219	21	61	99.5
A8	600	223	27,061	11	25	99.8
A9	1000	371	49,871	6	14	99.8

a: Based on an infusion duration of 60 minutes.

b: NOAEL is 100 mg/kg IV from 1-month GLP toxicology-monkey study (C_{max} = 2,350 µg/mL and AUC = 680,000 µg/mL · h).

c: Based on maximum dose for a 70-kg human.

FIGURE 5

Projected Safety Margins for BMS-986325					Nonclinical Margins at FIH SAD Doses ^b				
Species/Study	NOAEL Dose mg/kg	Cmax µg/mL	AUC (0-168 h) µg•h/mL	Total AUC (4 weeks) ^a µg•h/mL	Parameter	IV			SC
						0.3 mg	1000 mg	300 mg	
Cynomolgus Monkey / 1-Month (qw)	100	2,350	170,000	680,000	Dose (mg/kg):	23,333	7	23	
					AUC:	1,192,982	14	80	
					Cmax:	26,111	6	55	
Phase 1 Clinical Trial					Predicted AUC(INF)				
Human Dose Projection		Dose mg		Predicted Cmax µg/mL		µg•h/mL			
SAD starting dose		0.3		0.09		0.57			
SAD maximum IV dose		1000		371		49,871			
SAD SC dose		300		43		8,567			

a: The AUC(0-168h) after the last weekly dose in monkeys was multiplied by 4 to normalize for the total dosing interval of 4 weeks of exposure (Total AUC[4 weeks]) in the 1-month toxicity study.

b: Based on maximum dose for a 70-kg human.