SUPPLEMENTAL MATERIALS

Antithrombotic effects of

combined protease-activated receptor-4 antagonism

and factor Xa inhibition
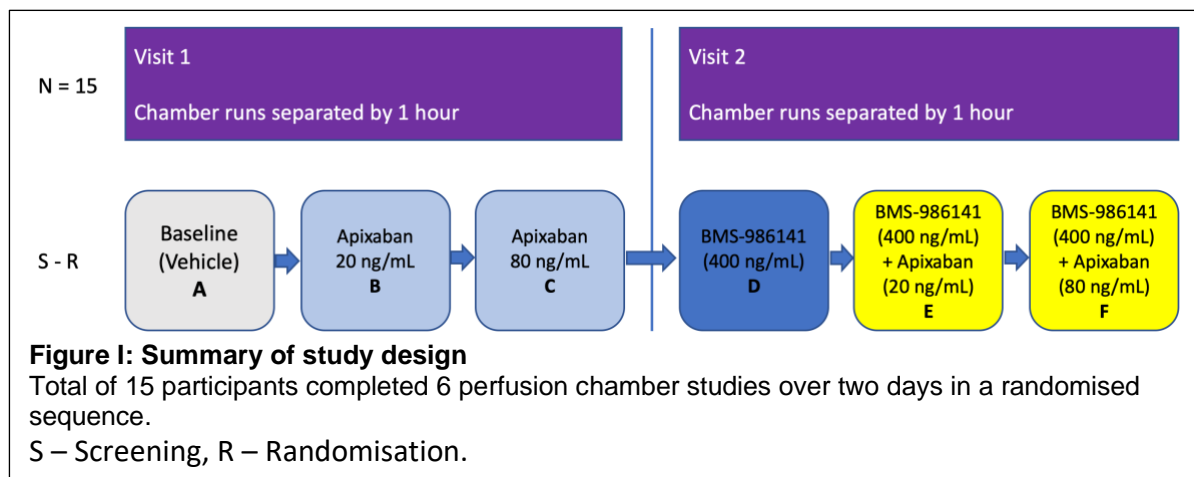
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Supplemental Figures

N = 15

Visit 1	Visit 2
Chamber runs separated by 1 hour	Chamber runs separated by 1 hour

S - R

Baseline (Vehicle) **A** → Apixaban 20 ng/mL **B** → Apixaban 80 ng/mL **C** → BMS-986141 (400 ng/mL) **D** → BMS-986141 (400 ng/mL) + Apixaban (20 ng/mL) **E** → BMS-986141 (400 ng/mL) + Apixaban (80 ng/mL) **F**

Figure I: Summary of study design
Total of 15 participants completed 6 perfusion chamber studies over two days in a randomised sequence.
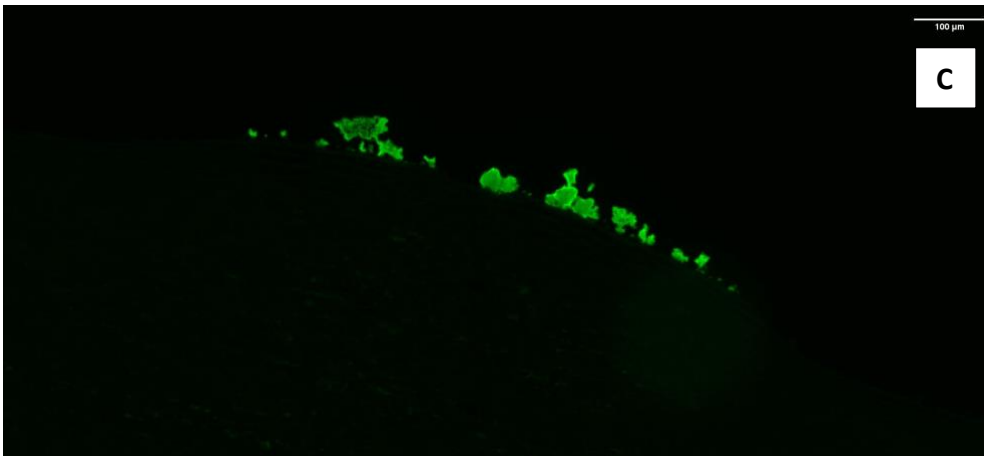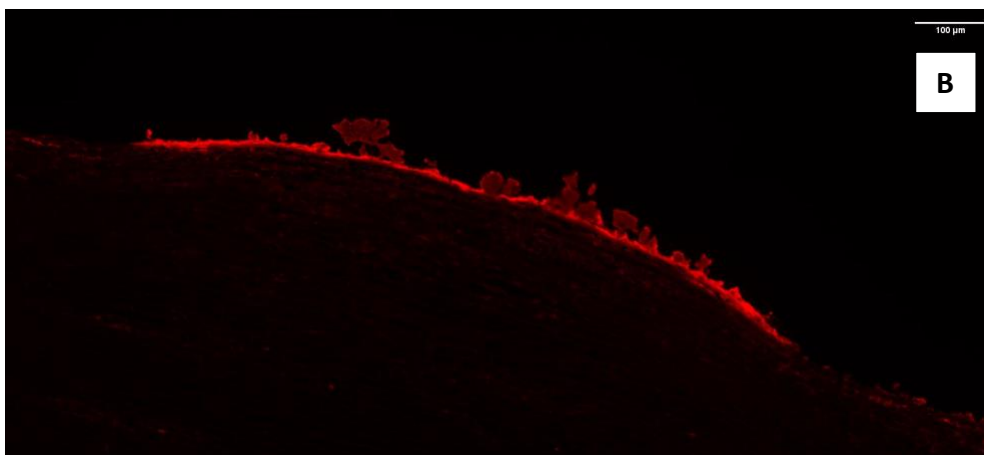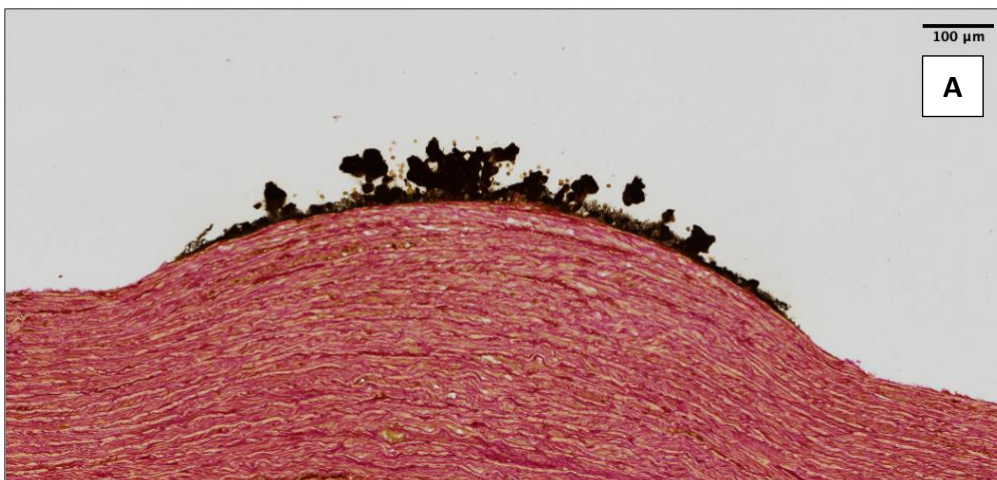S – Screening, R – Randomisation.

Figure II: Representative images of porcine aorta.
[A] Representative image of porcine aorta exposed to human blood with polyclonal goat antihuman fibrin antibody and CD61 monoclonal mouse antihuman antibody to allow quantification of total thrombus area.
[B] Representative image of porcine aorta exposed to human blood with polyclonal goat antihuman fibrin antibody and CD61 monoclonal mouse antihuman antibody to allow quantification of fibrin-rich thrombus area (counterstained with cyanine 3).
[C] Representative image of porcine aorta exposed to human blood with polyclonal goat antihuman fibrin antibody and CD61 monoclonal mouse antihuman antibody to allow quantification of platelet-rich thrombus area (counterstained with fluorescein isothiocyanate).
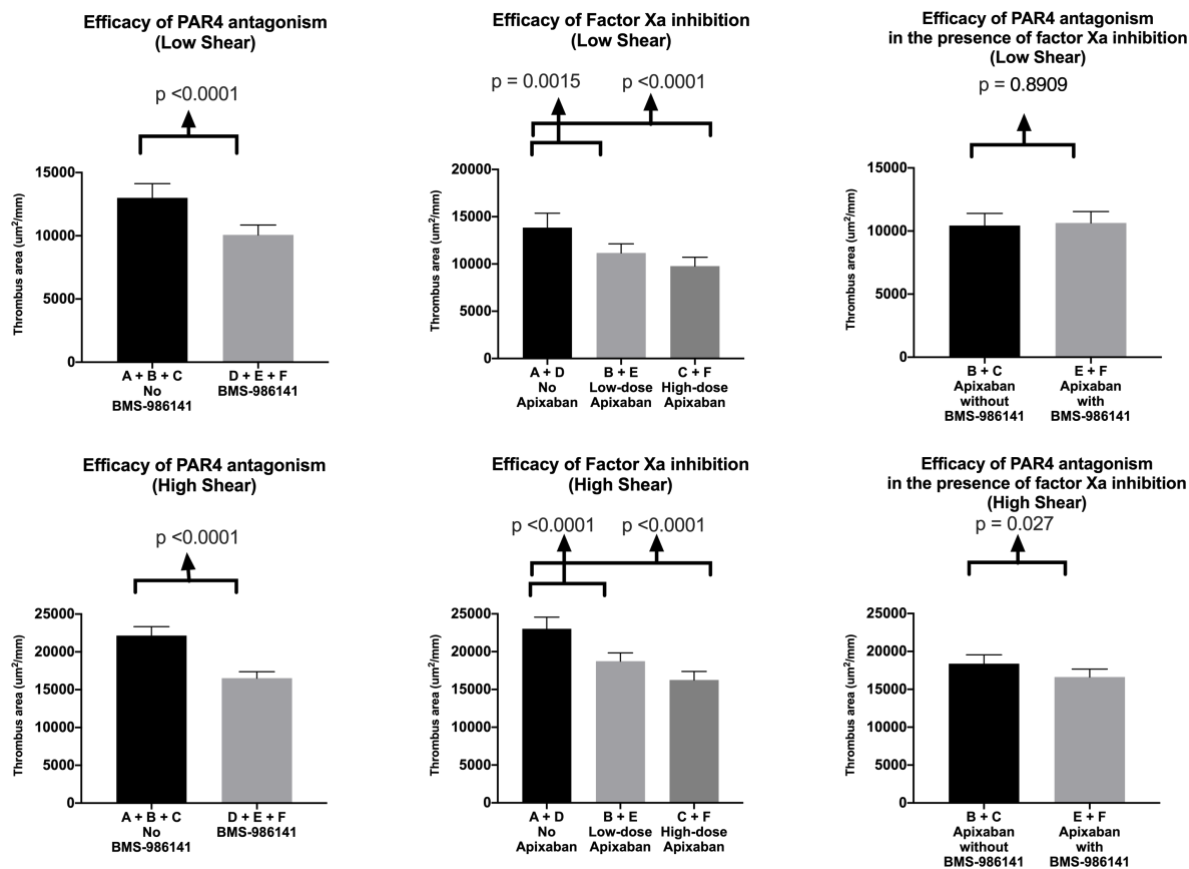
Figure III: Total Thrombus Area in the *Ex-Vivo* Perfusion Chamber

Efficacy of compounds at reducing total thrombus area in conditions of low-shear and high-shear stress. All compound(s) reduced total thrombus area at both high and low-shear stress conditions. The addition of BMS-986141 to apixaban led to further reduction in total thrombus area in conditions of high-shear stress.

(A)- Vehicle, (B)- low dose apixaban, (C)- high dose apixaban, (D)- BMS-986141, (E)- low-dose apixaban and BMS-986141, (F)- high-dose apixaban and BMS-986141.

Figure IV: Platelet-rich Thrombus Area in the *Ex-Vivo* Perfusion Chamber

Efficacy of compounds at reducing platelet-rich thrombus area in conditions of low-shear and high-shear stress. Compound(s) had no discernible effect in conditions of low shear. At high shear BMS-986141 but NOT apixaban, caused a reduction in platelet-rich thrombus area. The addition of BMS to apixaban caused further reductions in platelet-rich thrombus area.

(A)- Vehicle, (B)- low dose apixaban, (C)- high dose apixaban, (D)- BMS-986141, (E)- low dose apixaban and BMS-986141, (F)- high dose apixaban and BMS-986141.
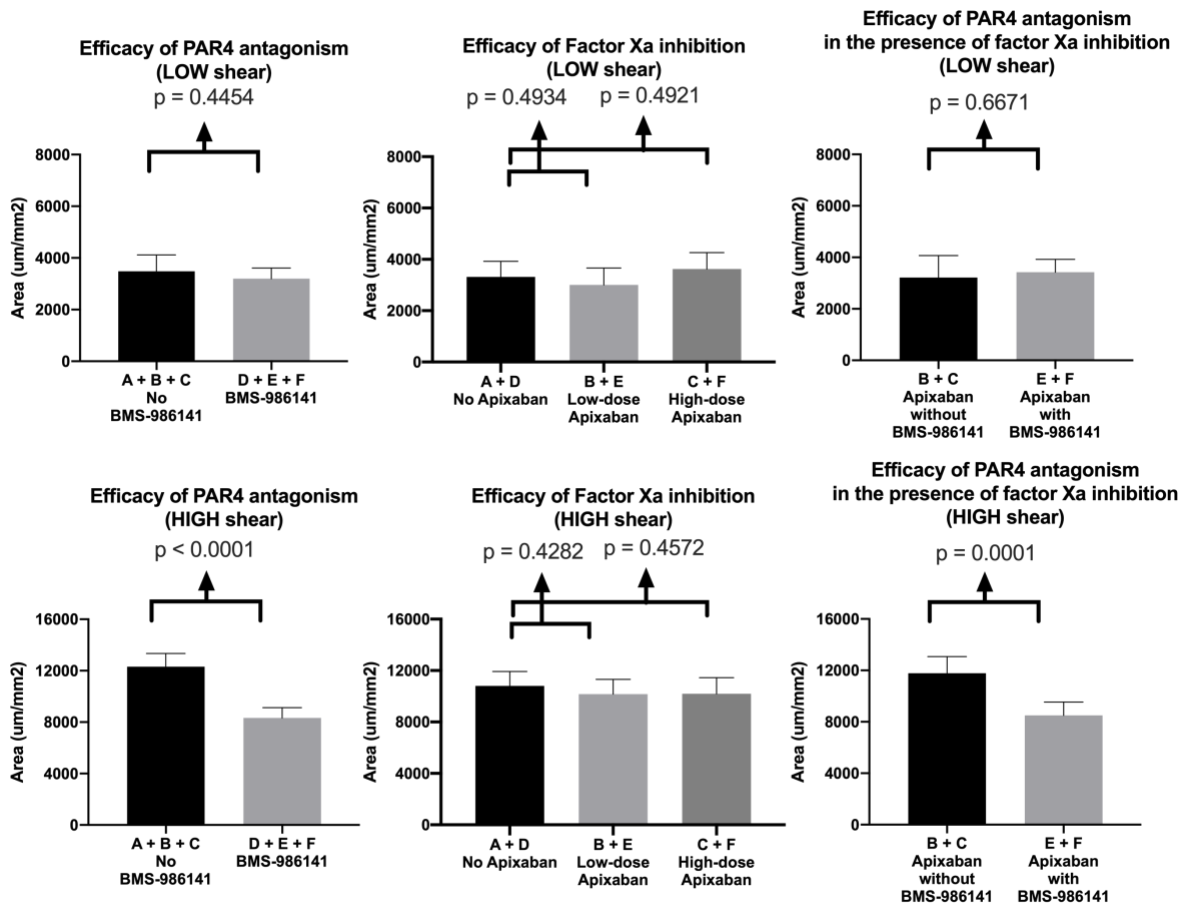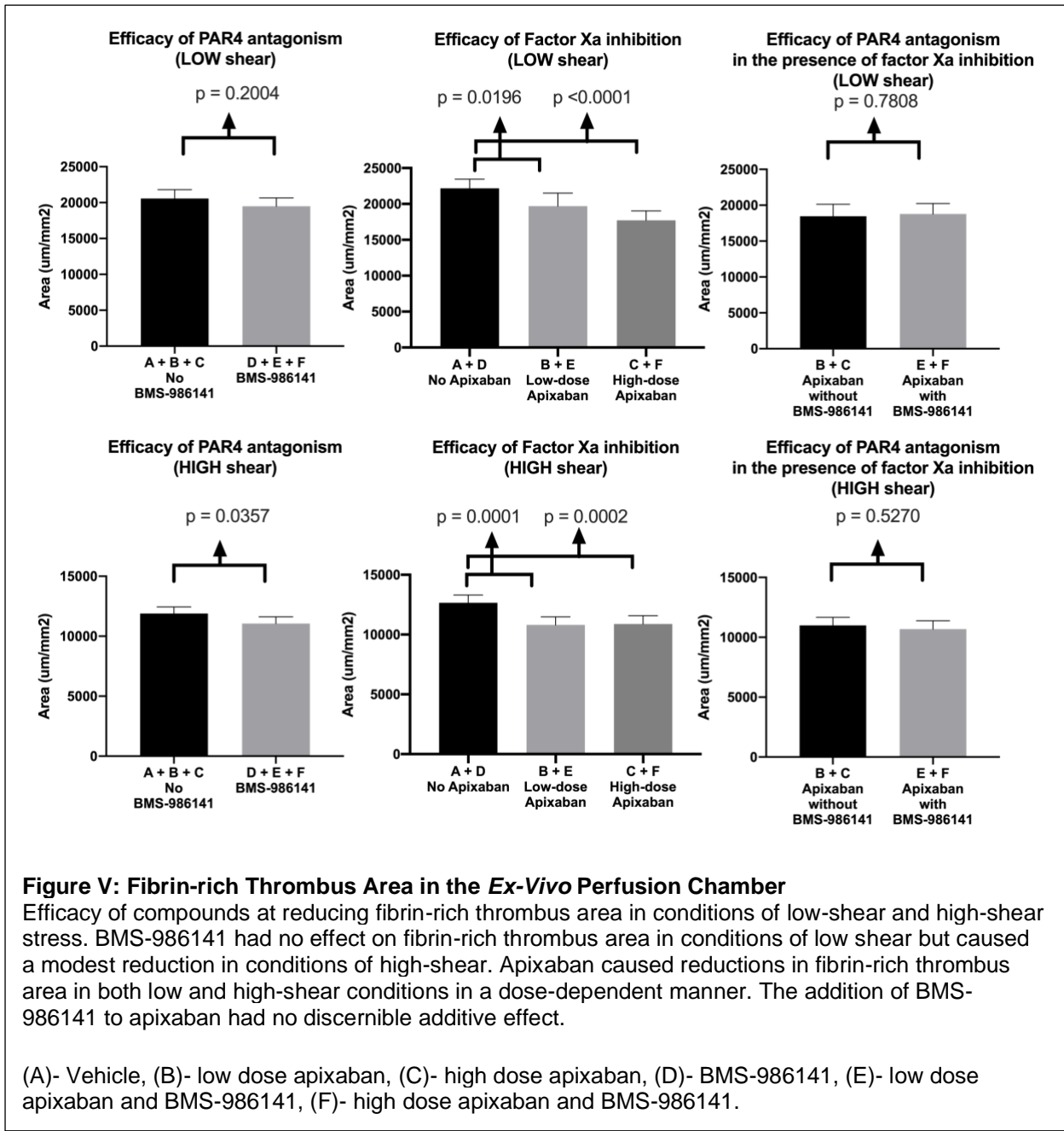
Figure V: Fibrin-rich Thrombus Area in the *Ex-Vivo* Perfusion Chamber
Efficacy of compounds at reducing fibrin-rich thrombus area in conditions of low-shear and high-shear stress. BMS-986141 had no effect on fibrin-rich thrombus area in conditions of low shear but caused a modest reduction in conditions of high-shear. Apixaban caused reductions in fibrin-rich thrombus area in both low and high-shear conditions in a dose-dependent manner. The addition of BMS-986141 to apixaban had no discernible additive effect.

(A)- Vehicle, (B)- low dose apixaban, (C)- high dose apixaban, (D)- BMS-986141, (E)- low dose apixaban and BMS-986141, (F)- high dose apixaban and BMS-986141.
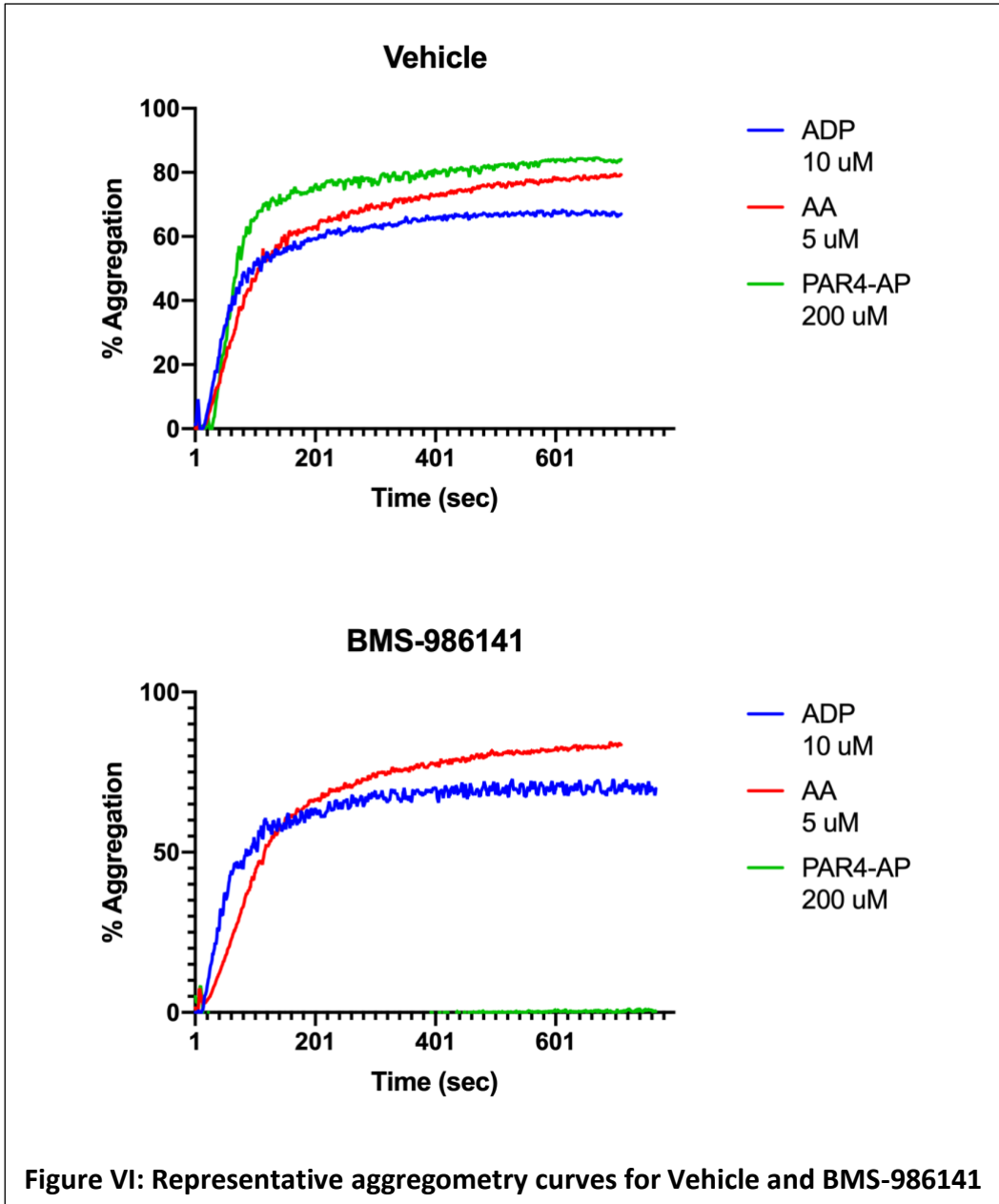
Figure VI: Representative aggregometry curves for Vehicle and BMS-986141

Supplemental Tables

Table I: Planned Perfusion Sequences						
No of participants	**Perfusion Sequence 1**	**Perfusion Sequence 2**	**Perfusion Sequence 3**	**Perfusion Sequence 4**	**Perfusion Sequence 5**	**Perfusion Sequence 6**
N= 3	A	B	C	D	E	F
N= 2	B	C	D	E	F	A
N= 2	C	D	E	F	A	B
N= 3	D	E	F	A	B	C
N= 2	E	F	A	B	C	D
N= 3	F	A	B	C	D	E
(A)- Placebo/vehicle, (B)- low-dose apixaban, (C)- high-dose apixaban, (D)- BMS-986141, (E)- BMS-986141 and low-dose apixaban, (F)- BMS-986141 and high-dose apixaban.						

Table II: Baseline characteristics of study participants	
Men	8 (53)
Age (years)	29.5±6.1
Body-mass index (kg/m2)	24.4±3.5
Haemoglobin (g/dL)	145±12
Platelet count (x10$_9$ /L)	260±41
Activated partial thromboplastin time (s)	29.8±4.1
Prothrombin time (s)	11.9±0.74
n (%); mean±standard deviation.	

Table III: Effluent Plasma Concentrations of Apixiban and BMS-986141		
Study Code	**Study Drug and Target Concentration**	**Plasma Concentration (ng/mL)**
A	Vehicle	-
B	Apixaban, 20 ng/mL	15.8±2.4
C	Apixaban, 80 ng/mL	72.3±18.7
D	BMS-986141, 400 ng/mL	285.0±75.1
E	Apixaban, 20 ng/mL	16.8±4.8
	BMS-986141, 400 ng/mL	289.9±68.2
F	Apixaban, 80 ng/mL	60.8±25.1
	BMS-986141, 400 ng/mL	293.3±76.6
mean±standard deviation		

Table IV: Platelet studies

Comparison of max platelet aggregation in presence of ADP

Comparators	Mean difference	95% Confidence Interval	Adjusted *P*-value
A vs B	-4.765	-26.32 to 16.79	0.6635
A vs C	-21.02	-42.57 to 0.5361	0.0559
A vs D	-19.97	-42.40 to 2.461	0.0807
A vs E	-0.3042	-21.49 to 20.89	0.9775
A vs F	-4.465	-25.65 to 16.72	0.6784

Comparison of max platelet aggregation in presence of AA

Comparators	Mean difference	95% Confidence Interval	Adjusted *P*-value
A vs B	13.08	-8.474 to 34.63	0.2331
A vs C	-17.36	-38.91 to 4.194	0.1139
A vs D	4.261	-18.17 to 26.69	0.7085
A vs E	2.981	-18.21 to 24.17	0.7819
A vs F	-0.9936	-22.18 to 20.20	0.9265

Comparison of max platelet aggregation in presence of PAR4-AP

Comparators	Mean difference	95% Confidence Interval	Adjusted *P*-value
A vs B	-10.93	-32.48 to 10.63	0.3189
A vs C	-7.576	-29.13 to 13.98	0.4893
A vs D	64.62	42.19 to 87.05	<0.0001
A vs E	61.43	40.24 to 82.62	<0.0001
A vs F	67.20	46.01 to 88.39	<0.0001

Comparison of p-selectin expression in presence of PAR4-AP

Comparators	Mean difference	95% Confidence Interval	Adjusted *P*-value
A vs D	470648	319694 to 621602	<0.0001
A vs E	462512	311558 to 613466	<0.0001
A vs F	481773	330819 to 632726	<0.0001
C vs E	-8136	-159089 to 142818	0.9144
C vs F	11125	-139829 to 162079	0.8831

Comparison of platelet-monocyte aggregates in presence of PAR4-AP

Comparators	Mean difference	95% Confidence Interval	Adjusted *P*-value
A vs D	35036	7706 to 62366	0.0127
A vs E	30586	3256 to 57916	0.0288
A vs F	36398	9068 to 63728	0.0098
C vs E	-4450	-31780 to 22880	0.7464
C vs F	1362	-25968 to 28692	0.9211

(A)- Placebo/vehicle, (B)- low-dose apixaban, (C)- high-dose apixaban, (D)- BMS-986141, (E)- BMS-986141 and low-dose apixaban, (F)- BMS-986141 and high-dose apixaban. ADP – Adenosine diphosphate, AA – arachidonic acid, PAR4-AP – Protease-Activated Receptor 4 agonist peptide

Table V: Total Thrombus Area				
Comparison of Total Thrombus Area (LOW-SHEAR STRESS)				
Comparators	**Percentage difference (%)**	**Mean difference (μm_2/mm)**	**95% Confidence Interval**	**Adjusted *P*-value**
A vs B	-33.71	5765	3521 to 8008	<0.0001
A vs C	-42.92	7340	5267 to 9414	<0.0001
A vs D	-44.44	7600	5411 to 9789	<0.0001
A vs E	-35.68	6101	3858 to 8344	<0.0001
A vs F	-42.67	7297	5175 to 9418	<0.0001
Efficacy of PAR4 antagonism *	-22.59	2937	1616 to 4259	<0.0001
Efficacy of low-dose apixaban †	-19.38	2685	1026 to 4344	0.0015
Efficacy of high-dose apixaban ‡	-29.39	4072	2515 to 5628	<0.0001
Efficacy of combination §	-0.9	94.52	-1553 to 1742	0.8909
Comparison of Total Thrombus Area (HIGH-SHEAR STRESS)				
A vs B	-32.13	9278	6924 to 11632	<0.0001
A vs C	-40.92	11816	9403 to 14228	<0.0001
A vs D	-43.44	12544	10152 to 14936	<0.0001
A vs E	-38.38	11081	8663 to 13499	<0.0001
A vs F	-46.52	13432	11019 to 15845	<0.0001
Efficacy of PAR4 antagonism *	-25.47	5644	4228 to 7059	<0.0001
Efficacy of low-dose apixaban †	-18.58	4277	2565 to 5989	<0.0001
Efficacy of high-dose apixaban ‡	-29.40	6767	5035 to 8499	<0.0001
Efficacy of combination §	-9.65	1774	18.02 to 3529	0.027

* [Vehicle + apixaban low-dose + apixaban high-dose] **VS** [BMS + BMS & low-dose apixaban + BMS & high-dose apixaban]

† [Vehicle + BMS] **VS** [low-dose apixaban + BMS & low-dose apixaban]

‡ [Vehicle + BMS] **VS** [high-dose apixaban + BMS & high-dose apixaban]

§ [Apixaban low-dose + Apixaban high-dose] **VS** [BMS & low-dose apixaban + BMS & high-dose apixaban].

(A)- Placebo/vehicle, (B)- low-dose apixaban, (C)- high-dose apixaban, (D)- BMS-986141, (E)- BMS-986141 and low-dose apixaban, (F)- BMS-986141 and high-dose apixaban.

Table VI: Platelet-rich and Fibrin-rich Thrombus Area								
	Comparison of Platelet-rich Thrombus Area (LOW-SHEAR STRESS)				Comparison of Fibrin-rich Thrombus Area (LOW-SHEAR STRESS)			
Comparators	Percentage difference (%)	Mean difference ($\mu m_2/mm$)	95% Confidence Interval	Adjusted *P*-value	Percentage difference (%)	Mean difference ($\mu m_2/mm$)	95% Confidence Interval	Adjusted *P*-value
A vs B	-27.01	1038	-302.2 to 2378	0.1286	-6.42	745.9	-721.4 to 2213	0.3181
A vs C	-7.44	285.4	-975.4 to 1546	0.6565	-31.62	3673	2307 to 5039	<0.0001
A vs D	-28.83	1108	-90.44 to 2306	0.0699	-10.23	1188	-124.6 to 2501	0.0759
A vs E	-18.21	699.5	-503.7 to 1903	0.2537	-21.52	2500	1169 to 3831	0.0003
A vs F	-4.37	167.7	-1004 to 1339	0.7784	-16.96	1970	663.2 to 3277	0.0032
Efficacy of PAR4 antagonism *	-8.15	284.1	-445.4 to 1014	0.4454	-5.32	546.9	-290.7 to 1385	0.2004
Efficacy of low-dose apixaban †	-9.41	311.8	-588.1 to 1212	0.4934	-11.10	1231	197.4 to 2264	0.0196
Efficacy of high-dose apixaban ‡	-9.26	-307.1	-1172 to 557.9	0.4921	-20.05	2222	1229 to 3216	<0.0001
Efficacy of combination §	-6.34	-203.4	-1131 to 724.6	0.6671	-1.65	-152.8	-1230 to 924.3	0.7808
	Comparison of Platelet-rich Thrombus Area (HIGH-SHEAR STRESS)				Comparison of Fibrin-rich Thrombus Area (HIGH-SHEAR STRESS)			
A vs B	-11.82	1558	-627.3 to 3743	0.1621	-19.57	2616	1321 to 3911	<0.0001
A vs C	-9.35	1233	-989.6 to 3456	0.2765	-15.82	2114	808.2 to 3420	0.0015
A vs D	-39.32	5185	2991 to 7379	<0.0001	-11.71	1565	264.8 to 2865	0.0184
A vs E	-34.49	4547	2335 to 6760	<0.0001	-18.82	2516	1212 to 3819	0.0002
A vs F	-36.54	4819	2581 to 7056	<0.0001	-21.32	2850	1544 to 4156	<0.0001
Efficacy of PAR4 antagonism *	-32.33	3979	2653 to 5306	<0.0001	-7.01	834.1	55.91 to 1612	0.0357
Efficacy of low-dose apixaban †	-5.96	643.3	-955.8 to 2242	0.4282	-14.70	1860	917.8 to 2803	0.0001
Efficacy of high-dose apixaban ‡	-5.83	629.2	-991.8 to 2250	0.4572	-14.04	1776	828.6 to 2724	0.0002
Efficacy of combination §	-27.83	3280	1622 to 4938	0.0001	-2.85	313.1	-657.3 to 1284	0.5270

* [Vehicle + apixaban low-dose + apixaban high-dose] **VS** [BMS + BMS & low-dose apixaban + BMS & high-dose apixaban], † [Vehicle + BMS] **VS** [low-dose apixaban + BMS & low-dose apixaban], ‡ [Vehicle + BMS] **VS** [high-dose apixaban + BMS & high-dose apixaban], § [Apixaban low-dose + Apixaban high-dose] **VS** [BMS & low-dose apixaban + BMS & high-dose apixaban]. (A)- Placebo/vehicle, (B)- low-dose apixaban, (C)- high-dose apixaban, (D)- BMS-986141, (E)- BMS-986141 and low-dose apixaban, (F)- BMS-986141 and high-dose apixaban.

Major Resources Table:

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration
Fibrinogen (Rabbit)	DAKO	A0080	1.2 μg/mL (1:5000)
CD61 (Mouse)	Dako	M0753	1.28 μg/mL 1:50
Normal goat serum	Bio sera	GO605/500	1:5
Goat anti Rabbit Peroxidase	Abcam	Ab7171	1:1000
Goat anti-Mouse Peroxidase	Abcam	Ab6823	1:1000
DAPI	Sigma-Aldrich	D9542	5 μg/mL 1:500
Tyrimide Cy3	Perkin Elmer	NEL 744B001KT	1:50
Tyrimide FITC	Perkin Elmer	NEL 741B00KT	1:50

Other

Description	Source / Repository
Proteinase k	DAKO: S3020
Refine kit	Leica biosystems: DS9800