METHODS AND RESULTS

Radiosynthesis of [^{11}C]-GSK215083 at Dresden

For all radiosyntheses at Dresden, the methylation agent, ^{11}C-methyliodide was used. ^{11}C-Methyliodide was prepared by catalytic reduction of ^{11}C-carbon dioxide to ^{11}C-methanol with lithium aluminum hydride followed by iodination with hydriodic acid. ^{11}C-GSK215083 was prepared by reacting the free base of the corresponding desmethyl precursor (1mg) with [^{11}C]-methyliodide in the presence of 2,2,6,6,-tetramethylpiperidine (10µl) at 80^{o}C in a 2:1 mixture of methanol:acetonitrile (300µl). After 5 minutes of heating, the reaction mixture was diluted with a solution of the mobile phase (500µl) and injected onto a semi-preparative column (SphereClone ODS(2) C-18, 250x10mm (Phenomenex); mobile phase, acetonitrile:70mM NaH_2PO_4 (60:40); flow rate, 9ml/min) for high-performance liquid chromatography (HPLC) purification. Fractions between 10 and 12 minutes were combined, evaporated to dryness and reformulated using saline. The reformulated product was analysed for radiochemical purity and specific activity by radio-HPLC (SphereClone C-18, 250x4.6mm; acetonitrile:70mM NaH_2PO_4 (70:30); flow rate 2ml/min; retention time, 4.8 minutes).

Blood samples for PK analysis of SB742457 at Dresden

For subjects in Group 2, blood samples were collected prior to and on completion of each scan when SB742457 was administered. The fifth and sixth subjects this group had two additional samples for SB742457 collected in the third scan session only, pre- and post-scan.

For subjects in Group 3, a pharmacokinetic blood sample was collected prior to dosing with SB742457 on days when PET scans were conducted. On the last SB742457 dosing day, additional blood samples were collected for assay of SB742457 and its main metabolites (Pre-dose, 1, 2, 4, 5 (pre PET scan), 6.5 (end of PET scan), 9, 12 and 24 hour after dosing with SB742457).

Approximately 1 ml of whole blood was collected into EDTA tubes and immediately stored on ice. Within 30 minutes of collection, tubes were centrifuged (2000 rpm, 5 minutes at 4°C) and the harvested plasma stored at -70°C pending shipment for drug analysis. Samples were analysed for SB742457 and its metabolites using a validated analytical method based on protein

precipitation, followed by HPLC/MS/MS analysis. Data are presented in Supplemental Tables 1 and 2 below.

SUPPLEMENTAL TABLE 1: **Group 2 - Listing of Pharmacokinetic Parameters for SB742457**

Subject	Treatment (a)	Cave Scan 2	Cave Scan 3
	[mg]	[ng/ml]	[ng/ml]
201	175 SD		228.4
202	175 SD	300.4	
204	175 SD		231.2
205	175 SD (b)	297.4	4.8
206	175 SD (b)	258.9	9.2
1203	175 SD	214.9	

(a) SD=Single Dose

(b) No SB742457 Dose given on the day of Scan 3. Scan 3 was 1 week after Scan 2.

SUPPLEMENTAL TABLE 2: **Group 3 - Listing of Pharmacokinetic Parameters for SB742457**

Subject	Treatment (a)	AUCτ-ss	Cmax-ss	tmax-ss	Cave Sc 2	Cave Sc 3
	[mg]	[hr*ng/ml]	[ng/ml]	[hr]	[ng/ml]	[ng/ml]
301	35 RD	2223	111	1.05		
302	35 RD	2778	158	3.98	122.4	113.1
303	35 RD	2054	121	4.00	110.2	105.6
304	35 RD	2416	167	2.00	90.9	94.7
305	15 RD	804	47	3.97	38.8	38.4
306	15 RD	826	53	5.00	46.9	51.3
307	3 RD	368	22	4.03	17.6	17.7
308	3 RD	243	15	4.17	11.9	12.1

(a) RD=Repeat Dose

In Vivo Human PET Studies – CAMH

Metabolite Analysis Method

Blood was collected in heparinised tubes and centrifuged (5 minutes at 2000x*g*), and isolated plasma used directly for HPLC analysis. HPLC analysis of plasma was performed by minor modifications of the method described by Hilton and coworkers (2000; (*1)*). Briefly, samples were loaded onto a 5mL HPLC injector loop (Valco, Texas) and injected onto a small capture column (20x4.6mm) packed in-house with OASIS^{TM} HLB 30µm, (Waters, NJ). The capture column was eluted with 1% aqueous acetonitrile (2mL/min; 3 min) then back-flushed (30% acetonitrile/70% H_2O + 0.1N ammonium formate, pH 4) onto a Phenomenex 10µ Luna C18 column (250x4.6mm). Both column effluents were monitored through a flow detector (Bioscan Flow-Count) operated in coincidence mode. All radioactivity data were corrected for physical decay and integrated using a PC.

In Vivo Human PET Studies – Dresden

Study AZ313943 Safety Data

Safety monitoring in Study AZ3103943 comprised the following:

Screening and follow-up procedures (7–14 days after the last dose) included medical history (screening only), physical examination, clinical laboratory examinations, 12-lead ECG, vital signs (blood pressure and pulse rate). On study days, 12-lead ECG, vital signs and clinical laboratory assessments were conducted prior to PET scans; lead II continuous ECG and blood pressure monitoring were conducted during the PET scanning procedure. During the repeat dosing period, 12-lead ECGs and vital signs were measured prior to each dose administration and 24 hours after the last dose. Adverse events were collected throughout the study period.

Across the study, no serious adverse events were reported and there were no withdrawals from the study. There were no clinically significant changes in ECGs, vital signs or clinical laboratory values, with the exception of a single report of mild tachycardia in a subject receiving ketanserin and mild neutropenia in a subject receiving SB742457 175/35 mg. Adverse events reported during the study were all rated as either mild or moderate in severity and are presented below in Supplemental Tables 3 and 4.

SUPPLEMENTAL TABLE 3: AEs Reported in Groups 1 and 2 by Treatment Group

Adverse Events, n (%)	**GSK215083** **N=10**	**Ketanserin[(a)]** **N=4**	**175mg SB742457[1]** **N=6**
Any AE	**2 (20)**	**3 (75)**	**1 (17)**
Headache	1 (10)	1 (25)	0
Dizziness	0	1 (25)	0
Paraesthesia	0	1 (25)	0
Haematoma	1 (10)	1 (25)	0
Tachycardia	0	1 (25)	0
Abdominal pain	0	0	1 (17)
Fatigue	0	1 (25)	0
Arthropod bite	1 (10)	0	0
Benign breast neoplasm	0	1 (25)	0
Any AE related to investigational product	**0**	**1 (25)**	**0**
Tachycardia	0	1 (25)	0
Dizziness	0	1 (25)	0

[(a)] GSK215083 administered 2 hours after ketanserin dose and 5 hours after SB742457 dose

SUPPLEMENTAL TABLE 4: AEs Reported in Group 3 by Treatment Group (Safety Population)

Adverse Events, n (%)	GSK215083 N=8	175/35mg SB742457 N=4	70/15mg SB742457 N=2	15/3mg SB742457 N=2
Any AE	**1 (13)**	**3 (75)**	**1 (50)**	**1 (50)**
Headache	1 (13)	1 (25)	0	1 (50)
Dizziness	0	1 (25)	0	0
Haematoma	0	1 (25)	1 (50)	0
Neutropenia	0	1 (25)	0	0
Constipation	0	1 (25)	0	0
Bronchitis viral	1 (13)	0	0	0
Back pain	0	1 (25)	0	0
Insomnia	0	0	1 (50)	0
Rash	0	1 (25)	0	0
Any AE related to investigational product	**1 (13)**	**2 (50)**	**1 (50)**	**1 (50)**
Headache	1 (13)	1 (25)	0	1 (50)
Dizziness	0	1 (25)	0	0
Neutropenia	0	1 (25)	0	0
Constipation	0	1 (25)	0	0
Insomnia	0	0	1 (50)	0

^{11}C-GSK215083 Test-Retest at Dresden

Test-retest data from the four Dresden subjects scanned at Dresden are given in Supplemental Table 5 using the FRTM. Consistent intraclass correlation coefficient (ICC) values generated from the test-retest were low, 0.17 and 0.13 for the striatal regions and 0.03 for the frontal cortex. However, a heterogenous distribution pattern of ^{11}C-GSK215083 was observed throughout the brain, consistent with the subjects scanned at Toronto.

SUPPLEMENTAL TABLE 5: Test-Retest Data

Subject Number	Scan Number	FRTM BP values		
		Caudate	**Putamen**	**Frontal cortex**
Subject 1	Scan 1	0.504 (66.88%)	0.880 (1.95%)	0.265 (0.72%)
	Scan 2	0.841	0.897	0.263
Subject 2	Scan 1	0.795 (20.85%)	1.145 (14.64%)	0.279 (26.36%)
	Scan 2	0.961	0.977	0.206
Subject 3	Scan 1	1.186 (36.19%)	1.523 (33.64%)	0.351 (5.22%)
	Scan 2	1.615	2.035	0.370
Subject 4	Scan 1	*Poor fit*	4.904 (73.05%)	0.338 (6.13%)
	Scan 2	*Poor fit*	1.321	0.359
Mean±SD		**0.98±0.38**	**1.71±1.35**	**0.30±0.06**
ICC: ***((Scan1-Scan2)/Scan1+Scan2)*** ***(Mean±SD)***		**0.17±0.08**	**0.13±0.31**	**0.03±0.09**

Values in parentheses=percent change between each pair ((scan1-scan2/scan1)*100)

REFERENCES

(1) Hilton J, Yokoi F, Dannals RF, Ravert HT, Szabo Z, Wong DF. Column-switching HPLC for the analysis of plasma in PET imaging studies. Nucl Med Biol. 2000;27:627-630.