Supplementary materials

Supplementary results

Single-agent BEZ235

Metabolic responses by FDG-PET

- The percent change from baseline in the maximum standardized uptake values (SUVmax) of 18-fluoro deoxyglucose (18-FDG) was assessed by FDG-PET in cycle 1 and at the end of cycle 2. In cycle 1, 13 patients out of the 26 evaluable patients had a decrease in SUVmax (50%; Supplementary Figure 1A), including 8 who had a partial metabolic response (PMR, defined as ≥ 25% decrease from baseline in SUVmax in the absence of new lesions). Of these 8 patients, 3 had SD, 3 had progressive disease (PD), and 2 had unknown overall tumor response by RECIST v1.0. In addition, 3 of the 8 patients who had PMR had a PI3K pathway alteration: PTEN null/low expression was observed in 1 patient and *PIK3CA* mutation was observed in 2 patients (Supplementary Figure I A).
- At the end of cycle 2, 5 of the 16 evaluable patients had a decrease in SUVmax (31.3%; Supplementary Figure IA), including 4 patients with PMR, all of whom had SD as overall tumor response by RECIST v1.0. Of the 4 patients with PMR, 1 had a PI3K pathway alteration with a PTEN null/low expression (Supplementary Figure IA).

BEZ235 in combination with trastuzumab

Metabolic responses by FDG-PET

- Change in SUVmax by FDG-PET was assessed at the end of cycle 2. A decrease in SUVmax was observed in 5 of 8 evaluable patients (62.5%; Supplementary Figure IB), including 3 patients with PMR. Of these 3, 1 patient had a PR and two patients had SD as best overall response by RECIST v1.0.

For the 3 patients with PMR, 1 patient with PMR had a PI3K pathway alteration by central assessment (*PIK3CA* mutation H1047R in Exon 20; Supplementary Figure IB); the 2 additional patients were enrolled on the basis of locally assessed PI3K pathway alterations (*PIK3CA* mutation, PTEN null/low expression)

Supplementary Table 1A Summary of primary PK parameters for single-agent BEZ235 HGC formulation

BEZ235 cohort	Dose level	PK parameter (unit)	Cycle 1, Day 1	Cycle 1, Day 8	Cycle 1, Day 28
HGC fast (N = 41)	10 mg/day (N = 3)	$AUC_{(0-24)}$ (h*ng/mL)	21.71 (2)	33.13 (3)	26.51 (3)
		C_{max} (ng/ml)	13.30 (3)	9.29 (3)	5.27 (3)
		$t_{1/2}$ (h)	–	–	–
		T_{max} (h)	2.00 (3)	3.00 (3)	4.00 (3)
	25 mg/day (N = 6)	$AUC_{(0-24)}$ (h*ng/mL)	20.98 (6)	24.71 (6)	18.92 (3)
		C_{max} (ng/ml)	12.15 (6)	10.54 (6)	12.13 (3)
		$t_{1/2}$ (h)	–	–	–
		T_{max} (h)	1.00 (6)	1.30 (6)	1.25 (3)
	50 mg/day (N = 4)	$AUC_{(0-24)}$ (h*ng/mL)	70.75 (4)	80.82 (4)	63.40 (4)
		C_{max} (ng/mL)	16.35 (4)	21.05 (4)	15.81 (4)
		$t_{1/2}$ (h)	–	–	–
		T_{max} (h)	2.29 (4)	2.31 (4)	1.55 (4)
	100 mg/day (N = 6)	$AUC_{(0-24)}$ (h*ng/mL)	54.94 (5)	149.85 (6)	125.06 (5)
		C_{max} (ng/mL)	14.10 (6)	37.80 (6)	28.15 (6)
		$t_{1/2}$ (h)	–	–	–
		T_{max} (h)	2.54 (6)	2.49 (6)	1.34 (6)

BEZ235 cohort	Dose level	PK parameter (unit)	Cycle 1, Day 1	Cycle 1, Day 8	Cycle 1, Day 28
	200 mg/day (N = 5)	$AUC_{(0-24)}$ (h*ng/mL)	534.17 (5)	209.76 (5)	379.96 (4)
		C_{max} (ng/mL)	71.50 (5)	51.20 (5)	43.40 (4)
		$t_{1/2}$ (h)	–	–	–
		T_{max} (h)	2.00 (5)	2.00 (5)	3.61 (4)
	300 mg/day (N = 6)	$AUC_{(0-24)}$ (h*ng/mL)	213.92 (5)	536.27 (6)	437.47 (4)
		C_{max} (ng/mL)	15.80 (6)	66.95 (6)	144.00 (5)
		$t_{1/2}$ (h)	–	–	–
		T_{max} (h)	3.02 (6)	3.50 (6)	3.77 (5)
	400 mg/day (N = 11)	$AUC_{(0-24)}$ (h*ng/mL)	260.43 (10)	202.14 (9)	1201.96 (8)
		C_{max} (ng/mL)	32.50 (11)	42.60 (10)	183.15 (8)
		$t_{1/2}$ (h)	–	–	–
		T_{max} (h)	2.97 (11)	2.58 (10)	5.00 (8)
HGC fed (N = 18)	300 mg/day (N = 6)	$AUC_{(0-24)}$ (h*ng/mL)	680.39 (6)	3208.65 (6)	5781.64 (4)
		C_{max} (ng/mL)	120.50 (6)	418.00 (6)	301.00 (5)
		$t_{1/2}$ (h)	–	–	–
		T_{max} (h)	3.98 (6)	3.95 (6)	6.00 (5)
	400 mg/day (N = 3)	$AUC_{(0-24)}$ (h*ng/mL)	544.65 (3)	1766.88 (2)	406.71 (3)
		C_{max} (ng/mL)	94.70 (3)	50.10 (3)	40.80 (3)
		$t_{1/2}$ (h)	–	–	–

BEZ235 cohort	Dose level	PK parameter (unit)	Cycle 1, Day 1	Cycle 1, Day 8	Cycle 1, Day 28
		T_{max} (h)	4.50 (3)	3.00 (3)	2.08 (3)
	700 mg/day (N = 5)	$AUC_{(0-24)}$ (h*ng/mL)	1253.47 (4)	11380.24 (4)	4385.64 (4)
		C_{max} (ng/mL)	116.00 (5)	926.00 (5)	421.75 (4)
		$t_{1/2}$ (h)	–	–	–
		T_{max} (h)	4.02 (5)	4.17 (5)	4.63 (4)
	1100 mg/day (N = 4)	$AUC_{(0-24)}$ (h*ng/mL)	280.92 (3)	931.22 (4)	604.35 (1)
		C_{max} (ng/mL)	275.90 (4)	100.35 (4)	46.10 (1)
		$t_{1/2}$ (h)	–	–	–
		T_{max} (h)	7.22 (4)	4.50 (4)	4.00 (1)

HGC hard gelatine capsule; *PK* pharmacokinetics;

All data shown as median (n).

Supplementary Table 1B Summary of primary PK parameters for single-agent BEZ235 SDS formulation

BEZ235 cohort	Dose level	PK parameter (unit)	Cycle 1, Day 1	Cycle 1, Day 8	Cycle 1, Day 28
SDS capsule A (N = 22)	400 mg/day (N = 5)	$AUC_{(0-24)}$ (h*ng/mL)	2247.84 (2)	10201.24 (4)	19555.36 (2)
		C_{max} (ng/mL)	131.00 (5)	982.00 (5)	826.00 (4)
		$t_{1/2}$ (h)	3.72 (2)	4.20 (4)	2.61 (2)
		T_{max} (h)	4.00 (5)	4.00 (5)	5.00 (4)
	800 mg/day (N = 6)	$AUC_{(0-24)}$ (h*ng/mL)	2543.6 (3)	19769.65 (4)	25404.92 (2)
		C_{max} (ng/mL)	164.00 (6)	1000.00 (5)	1940.00 (4)
		$t_{1/2}$ (h)	21.20 (1)	11.87 (2)	4.11 (1)
		T_{max} (h)	6.00 (6)	6.00 (5)	4.00 (4)
	1000 mg/day (N = 11)	$AUC_{(0-24)}$ (h*ng/mL)	2906.34 (9)	11725.47 (8)	14087.89 (4)
		C_{max} (ng/mL)	309.00 (11)	929.50 (10)	701.00 (6)
		$t_{1/2}$ (h)	4.02 (6)	5.10 (2)	4.10 (2)
		T_{max} (h)	6.00 (11)	6.00 (10)	4.00 (6)
SDS sachet (N = 61)	800 mg/day (N = 6)	$AUC_{(0-24)}$ (h*ng/mL)	982.4 (6)	6959.56 (3)	5888.51 (3)
		C_{max} (ng/mL)	191.00 (6)	814.00 (3)	668.00 (3)
		$t_{1/2}$ (h)	6.72 (5)	6.67 (2)	4.67 (3)
		T_{max} (h)	1.53 (6)	4.00 (3)	2.03 (3)
	1000 mg/day (N = 10)	$AUC_{(0-24)}$ (h*ng/mL)	5685.26 (10)	19423.08 (8)	1357.59 (5)
		C_{max} (ng/mL)	489.50 (10)	1280.00 (9)	1280.00 (5)

BEZ235 cohort	Dose level	PK parameter (unit)	Cycle 1, Day 1	Cycle 1, Day 8	Cycle 1, Day 28
		$t_{1/2}$ (h)	5.01 (5)	9.68 (5)	5.89 (5)
		T_{max} (h)	5.04 (10)	4.07 (9)	4.00 (5)
	1200 mg/day (N = 24)	$AUC_{(0-24)}$ (h*ng/mL)	1895.69 (17)	13113.03 (18)	17996.82 (15)
		C_{max} (ng/mL)	194.50 (20)	1195.00 (20)	1335.00 (16)
		$t_{1/2}$ (h)	5.33 (13)	6.42 (12)	6.03 (12)
		T_{max} (h)	4.01 (20)	4.00 (20)	3.04 (16)
	1400 mg/day (N = 14)	$AUC_{(0-48)}$ (h*ng/mL)	5291.62 (9)	7622.28 (5)	18043.24 (5)
		C_{max} (ng/mL)	441.00 (13)	1180.50 (6)	1010.00 (5)
		$t_{1/2}$ (h)	4.97 (6)	5.69 (3)	11.03 (4)
		T_{max} (h)	7.98 (13)	5.01 (6)	4.00 (5)
	1600 mg/day (N = 7)	$AUC_{(0-24)}$ (h*ng/mL)	2563.78 (7)	23828.46 (6)	13729.30 (4)
		C_{max} (ng/mL)	251.00 (7)	1900.00 (7)	977.00 (5)
		$t_{1/2}$ (h)	4.29 (4)	3.81 (3)	3.85 (3)
		T_{max} (h)	6.00 (7)	5.93 (7)	4.00 (5)
SDS capsule B (N = 11)	800 mg/day	$AUC_{(0-24)}$ (h*ng/mL)	1855.39 (10)	2960.60 (10)	4506.66 (8)
		C_{max} (ng/mL)	243.00 (11)	298.00 (10)	446.50 (8)
		$t_{1/2}$ (h)	4.85 (6)	5.53 (7)	7.08 (5)
		T_{max} (h)	4.00 (11)	5.00 (10)	4.00 (8)

All data shown as median (n). *PK* pharmacokinetic; *SDS* solid dispersion system.

Supplementary Table 1C Summary of primary PK parameters for BEZ235 SDS + trastuzumab

BEZ235 cohort	PK parameter (unit)	Cycle 1, Day 1	Cycle 1, Day 8	Cycle 1, Day 28
SDS capsule A 400 mg/day + T (N = 3)	$AUC_{(0-24)}$ (h*ng/mL)	5515.94 (1)	29681.34 (3)	8604.48 (2)
	C_{max} (ng/mL)	352.00 (3)	1630.00 (3)	1350.00 (3)
	$t_{1/2}$ (h)	-	12.64 (3)	6.48 (2)
	T_{max} (h)	6.05 (3)	6.00 (3)	4.00 (3)
SDS sachet 600 mg/day + T (N = 17)	$AUC_{(0-24)}$ (h*ng/mL)	4331.95 (15)	13419.26 (15)	7756.11 (14)
	C_{max} (ng/mL)	346.00 (16)	998.00 (15)	625.00 (15)
	$t_{1/2}$ (h)	5.12 (10)	5.89 (9)	5.73 (11)
	T_{max} (h)	4.23 (16)	4.07 (15)	4.00 (15)
SDS sachet 800 mg/day + T (N = 9)	$AUC_{(0-24)}$ (h*ng/mL)	6830.65 (8)	18805.82 (6)	13197.57 (3)
	C_{max} (ng/mL)	606.00 (10)	1185.00 (6)	1130.00 (3)
	$t_{1/2}$ (h)	5.31 (3)	8.00 (4)	6.77 (3)
	T_{max} (h)	6.96 (10)	4.04 (6)	4.00 (3)

All data shown as median (n).

PK pharmacokinetic; *SDS* solid dispersion system; *T* trastuzumab.

Supplementary Table 2 Summary of posterior distribution of DLT rates at time of MTD, dose determining analysis set

	Declared MTD	Posterior probabilities that Pr (DLT) is in interval					Quantiles		
		0-0.16	0.16-0.33	0.33-1	Mean	SD	2.5%	50%	97.5%
Single-agent BEZ235 SDS sachet	1200 mg/day	0.036	0.790	0.174	0.269	0.066	0.152	0.265	0.406
BEZ235 SDS sachet in combination with trastuzumab	600 mg/day + trastuzumab 2 mg/kg/day	0.261	0.609	0.130	0.224	0.091	0.079	0.214	0.43

DLT dose-limiting toxicity; *MTD* maximum tolerated dose; *Pr* probability; *SD* standard deviation; *SDS* solid dispersion system.

Supplementary Table 3 Patient disposition and deaths

	Single-agent SDS capsule A N = 22	Single-agent SDS sachet N = 61	All SDS + trastuzumab N = 30[a]
Primary reason for discontinuation			
Adverse event	1 (4.5)	14 (23.0)	5 (16.7)
Disease progression	20 (90.9)	44 (72.1)	22 (73.3)
Withdrew consent	1 (4.5)	3 (4.9)	3 (10)
Deaths			
Total	5 (22.7)	3 (4.9)	3 (10.0)
On-treatment	4 (18.2)	3 (4.9)	2 (6.7)

SDS solid dispersion system; *T* trastuzumab.

All data shown as n (%) unless stated otherwise.

[a]Of the 30 treated patients in this group, 1 patient had a serious adverse event of myocardial ischemia on cycle 1 day 1 and discontinued the study without receiving a dose of trastuzumab, and after only a single dose of 800 mg BEZ235 SDS sachet.

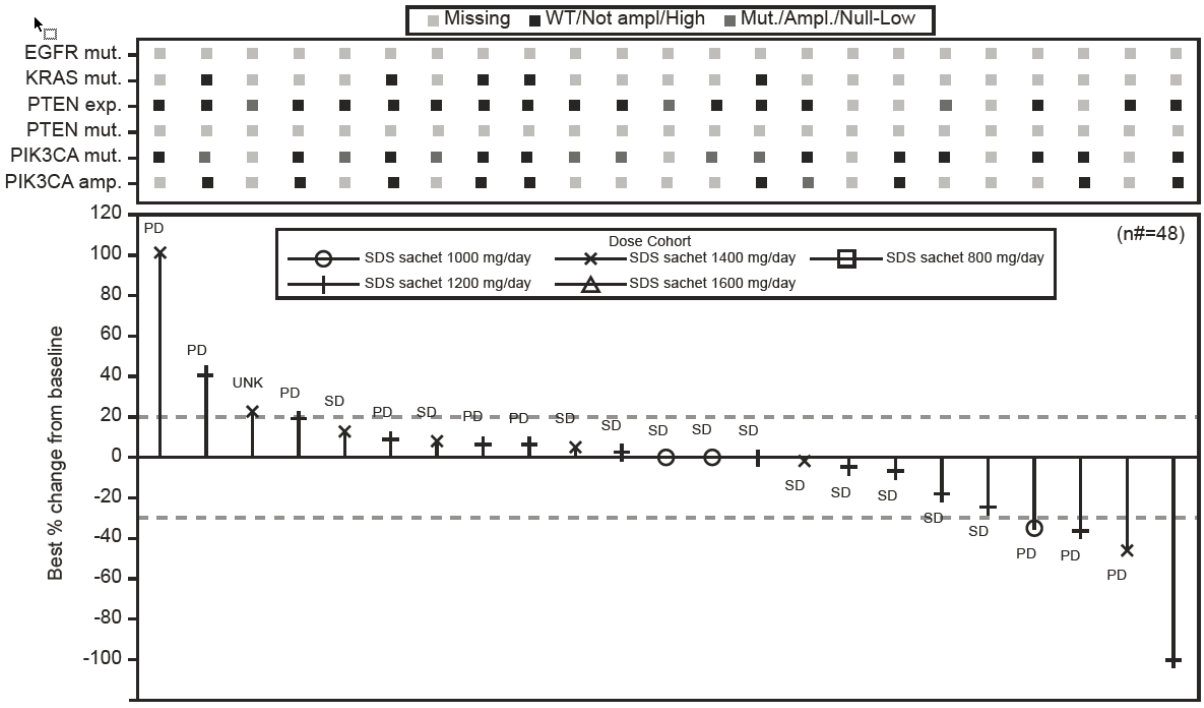
Supplementary Table 4 Biomarkers in tumor tissue from paired biopsies

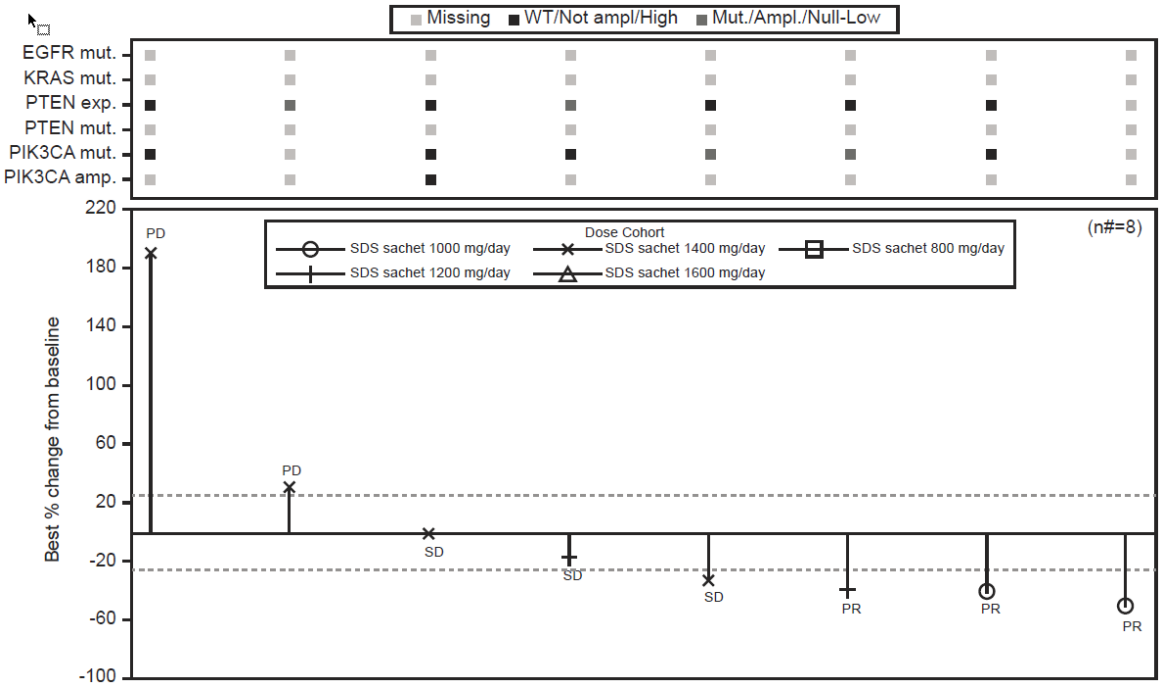
Patient	Dose cohort	Treatment duration	Reason for discon-tinuation	Timepoint	Change from baseline (%)								
					Ki67	pAKT	$pAKT^{473}$	pS6	$pS6^{253}$	PARP	p4EBP1	cyclin D1	pERK
A	SDS capsule A 800 mg/day	2 cycles	Disease progression	Cycle 2 day 28	−26.32	–	16.67	−26.92	–	−100.00	+26.67	–	–
B	SDS capsule A 1000 mg/day	3 cycles	Disease progression	Cycle 1 day 28	−66.67	−42.50	–	+86.85	–	–	0	−6.25	+47.37
				Cycle 3 day 28	−66.67	+40.00	–	+107.61	–	–	+33.33	+6.25	+50.00
C	SDS sachet 1200 mg/day	1.5 cycles	Adverse event	Cycle 1 day 28	–	–	–	–	–	–	0.00	–	–
D	SDS sachet 600 mg/day + trastuzumab	1.5 cycles	Disease progression	Cycle 1 day 28	–	+35.71	–	+2.33	+11.00	–	–	–	–

SDS solid dispersion system.

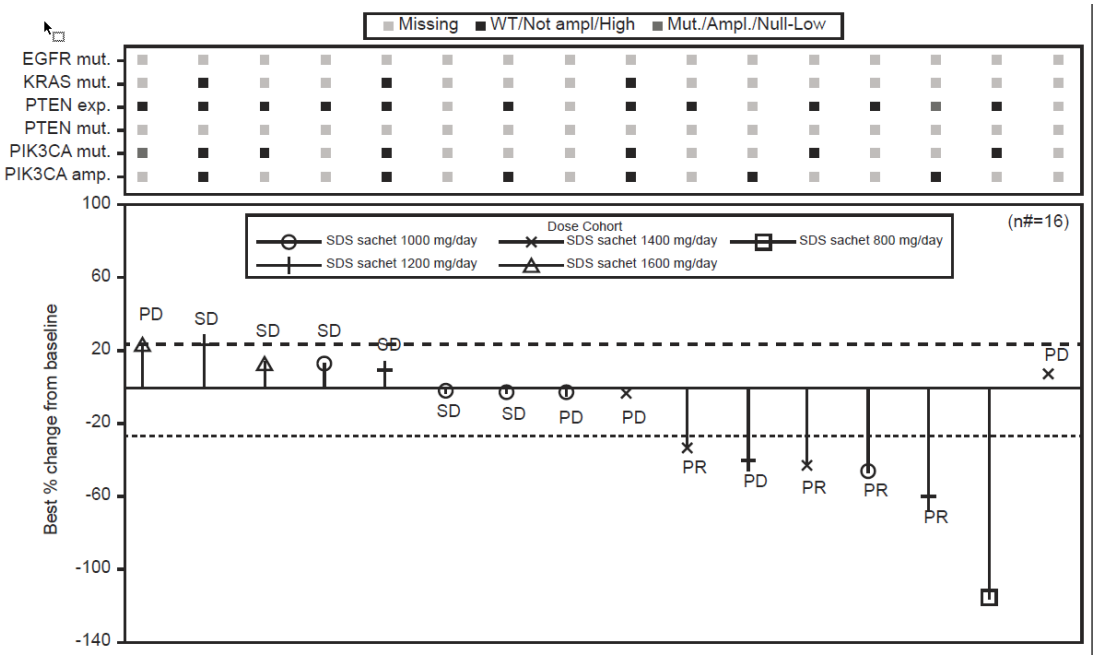
Supplementary Fig. 1 Percentage change from baseline by FDG-PET

A. Single-agent BEZ235 SDS sachet (To be re-drawn in high-res closer to submission)





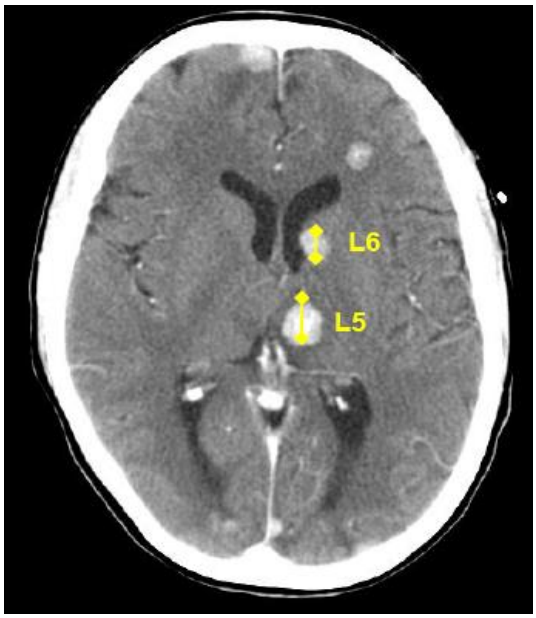
B. BEZ235 in combination with trastuzumab (To be re-drawn in high-res closer to submission)



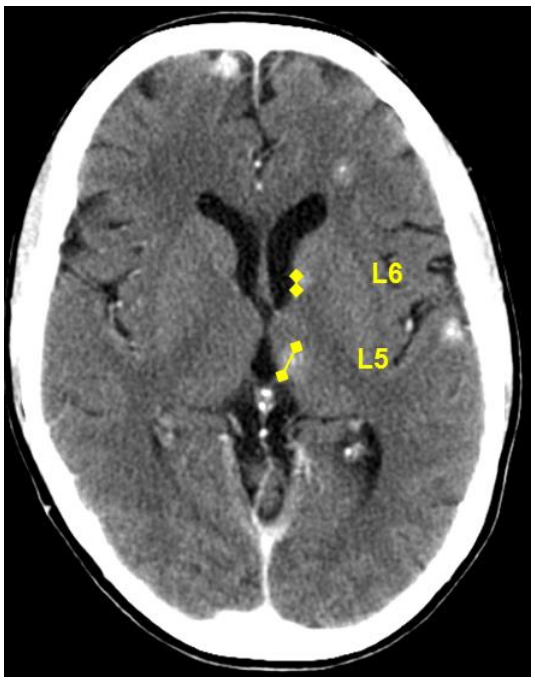
Supplementary Fig. 2 Scans at baseline and the end of cycle 4 for a patient with PR

Patient 0101-00802 had HER2+ aBC with locally assessed PI3K pathway alteration (*PIK3CA* mutation).

Baseline



Cycle 4 day 28



Assessment		Baseline, mm	C4D28, mm
BRAIN. Left thalamic nodule	L5	15.2	10.0
BRAIN. Left periventricular nodule	L6	11.0	7.0