Phase I studies of AZD1208, a proviral integration Moloney virus kinase inhibitor in solid and haematological cancers

SUPPLEMENTARY INFORMATION

Supplementary Table 1: Sampling schedule for pharmacokinetic analysis of AZD1208 in the AML and solid tumour trials

Blood sample		**Urine sample**	
AML dose-escalation study			
Cycle/Day	**Time**	**Cycle/Day**	**Time**
1/1	0 h pre-dose	1/1	Pre-dose
1/1	0.5 h post-dose	1/1	0–24 h
1/1	1 h post-dose		
1/1	1.5 h post-dose		
1/1	2 h post-dose		
1/1	3 h post-dose		
1/1	6 h post-dose		
1/1	8 h post-dose		
1/1	24 h post-dose		
1/7	0 h pre-dose		
1/14	0 h pre-dose	1/14	0–24 h
1/14	0.5 h post-dose		
1/14	1 h post-dose		
1/14	1.5 h post-dose		

1/14	2 h post-dose		
1/14	3 h post-dose		
1/14	6 h post-dose		
1/14	8 h post-dose		
1/14	24 h post-dose		
1/28	2 h post-dose		
Solid tumour dose-escalation study			
Cycle/Day	**Time**	**Cycle/Day**	**Time**
0/1	0 h pre-dose	0/1	Pre-dose
0/1	0.5 h post-dose	0/1–2	0–24 h
0/1	1 h post-dose		
0/1	2 h post-dose		
0/1	4 h post-dose		
0/1	6 h post-dose		
0/1	8 h dose-dose		
0/2	24 h post-dose	0/2–3	24–48 h
0/3	48 h post-dose	0/3–4	48–72 h
0/4	72 h post-dose		
1/2	0 h pre-dose		
1/5	0 h pre-dose		
1/8	0 h pre-dose		
1/8	2–6 h post-dose		
1/15	0 h pre-dose	1/15–16	0–24 h

1/15	0.5 h post-dose		
1/15	1 h post-dose		
1/15	2 h post-dose		
1/15	4 h post-dose		
1/15	6 h post-dose		
1/15	8 h post-dose		
1/16	24 h post-dose		
2/1	0 h pre-dose		
2/8	0 h pre-dose		
2/8	0.5 h post-dose		
2/8	1 h post-dose		
2/8	2 h post-dose		
2/8	4 h post-dose		
2/8	6 h post-dose		
2/8	8 h post-dose		
2/15	0 h pre-dose		
3+/1	0 h pre-dose		
Abbreviation: AML = acute myeloid leukaemia			

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Supplementary Table 2: Summary of AZD1208 plasma concentrations (pharmacokinetic analysis set) in the AML trial					
	AZD1208 120 mg *n*=3	**AZD1208 240 mg *n*=6**	**AZD1208 480 mg *n*=6**	**AZD1208 700 mg *n*=7**	**AZD1208 900 mg *n*=9**
Cycle 1, Day 1					
T_{max}, h					
Median	2.42	2.20	3.17	3.05	3.20
Min, max	2.00, 3.08	1.67, 3.08	2.00, 23.83	1.53, 8.00	2.97, 6.00
G_{mean} plasma concentration of AZD1208, ng ml (± SD)					
Pre-dose	NQ	76.9 (NC)	NQ	NQ	NQ
Post-dose, h					
0.5	85.8 (40.1, 183.9)	158.3 (14.4, 1740.3)	124.3 (67.6, 228.6)	386.8 (93.3, 1603.6)	251.5 (90.7, 697.2)

1	321.3 (187.8, 549.9)	540.4 (133.8, 2182.4)	573.0 (248.3, 1322.0)	883.8 (351.6, 2221.7)	921.4 (373.1, 2275.6)
1.5	774.5 (422.1, 1421.3)	1098.6 (620.9, 1943.7)	1243.2 (485.9, 3181.1)	1849.6 (783.5, 4366.4)	2106.0 (860.5, 5154.2)
2	779.9 (508.9, 1195.3)	1388.6 (1074.3, 1744.9)	1852.3 (781.9, 4387.6)	2640.6 (1438.6, 4847.0)	3553.2 (2352.6, 5366.6)
3	715.6 (415.5, 1232.6)	1299.5 (967.1, 1746.2)	2865.4 (1884.8, 4356.2)	3112.3 (1814.6, 5337.9)	3574.9 (1521.4, 8400.4)
6	546.6 (356.7, 837.6)	1004.2 (822.5, 1226.0)	2642.0 (1774.2, 3934.4)	2846.5 (2115.2, 3830.6)	4252.0 (2883.8, 6269.4)
8	481.6 (308.4, 752.3)	950.3 (825.3, 1094.2)	2249.0 (1520.2, 3327.1)	3284.7 (2253.3, 4788.2)	3882.9 (2438.1, 6184.0)
24	199.0 (75.6, 524.0)	844.5 (598.4, 1191.8)	2169.6 (1161.6, 4052.3)	2395.4 (1664.2, 5572.3)	3045.2 (1664.2, 5572.3)
Cycle 1, Day 14					

G_{mean} plasma concentration of AZD1208, ng ml (± SD)					
Pre-dose	1163.1 (335.5, 4032.0)	2106.9 (693.2, 6845.2)	2302.9 (541.6, 9792.3)	2513.6 (1057.2, 5976.4)	463.1 (444.2, 482.7)
Post-dose, h					
0.5	1758.9 (970.3, 3188.5)	2365.9 (976.3, 6694.0)	2887.4 (322.6, 25 841.7)	2029.2 (863.1, 4770.5)	640.6 (926.0, 955.4)
1	2050.2 (1514.6, 2775.2)	2556.4 (4058.6, 6516.6)	4956.2 (1164.7, 21 090.8)	2013.8 (1004.8, 4035.8)	1952.5 (1166.1, 3269.1)
1.5	2375.1 (1849.1, 3050.7)	5142.8 (4058.6, 6516.6)	5384.2 (2123.1, 13 654.8)	3963.7 (2236.9, 7023.5)	2747.1 (1719.7, 4388.3)
2	2216.3 (1388.5, 3537.7)	6153.8 (5277.1, 7175.8)	5818.8 (2320.7, 14 589.5)	4388.1 (2945.6, 6536.9)	3107.5 (1803.0, 5356.1)
3	2052.0 (1181.0, 3565.2)	3687.8 (1657.8, 8203.5)	5515.9 (1885.1, 16 139.7)	5521.1 (2560.5, 11 905.2)	3179.0 (1505.2, 6714.4)
6	1761.5 (1019.2, 3044.5)	3196.3 (1504.2, 6791.5)	5420.2 (1806.5, 16 262.9)	4452.1 (2126.6, 9320.8)	2320.5 (896.7, 6004.8)

8	1700.4 (974.4, 2967.5)	2735.5 (1203.4, 6218.4)	5248.1 (1804.2, 15 265.9)	4064.8 (2043.6, 8085.1)	2608.4 (2223.6, 3059.7)
24	1248.3 (485.6, 3208.8)	1818.9 (580.7, 5697.5)	3154.5 (406.4, 24 485.6)	2302.0 (643.6, 8233.0)	593.0 (174.0, 2021.0)

Abbreviations: AML = acute myeloid leukaemia; G_{mean} = geometric mean; NC = not calculable; NQ = non-quantifiable (below limit of quantification);

SD = standard deviation; T_{max} = median time to maximum plasma concentration

Supplementary Table 3: Summary of plasma concentration (ng ml) following single doses of AZD1208 (pharmacokinetic analysis set) in the solid tumour trial						
	AZD1208 120 mg *n*=3	**AZD1208 240 mg *n*=7**	**AZD1208 360 mg *n*=6**	**AZD1208 540 mg *n*=7**	**AZD1208 700 mg *n*=6**	**AZD1208 800 mg *n*=6**
Cycle 0, Day 1						
Time after dose, h						
Pre-dose	*n*=3 NC	*n*=7 NC	*n*=6 NC	*n*=7 NC	*n*=6 NC	*n*=2 NC
Post-dose, h						
0.25	*n*=3 1.793	*n*=7 12.83	*n*=6 29.51	*n*=7 26.29	–	–
0.5	*n*=3 20.28	*n*=7 257.5	*n*=6 343.5	*n*=7 200.2	*n*=6 139.2	*n*=6 222.3

1	*n*=3 156.2	*n*=7 668.4	*n*=5 1424	*n*=7 1053	*n*=6 691.7	*n*=6 925.6
1.5	*n*=3 696.5	*n*=7 1317	*n*=5 2509	*n*=7 2747	–	–
2	*n*=3 1064	*n*=7 1906	*n*=6 3412	*n*=7 4049	*n*=6 3514	*n*=6 2929
4	*n*=3 720.6	*n*=6 1531	*n*=6 3157	*n*=7 3160	*n*=6 4581	*n*=6 3844
6	*n*=3 681	*n*=7 1408	*n*=6 2501	*n*=7 3190	*n*=6 5091	*n*=6 2677
8	*n*=3 597.8	*n*=7 1456	*n*=6 2196	*n*=7 2957	*n*=6 4256	*n*=6 3463
24	*n*=3 459.4	*n*=7 1157	*n*=6 1649	*n*=7 2111	*n*=6 2957	*n*=6 2466

48	*n*=3 308.4	*n*=7 773.2	*n*=6 1361	*n*=7 1168	*n*=6 1717	*n*=6 1377
72	*n*=3 221.4	*n*=7 501.1	*n*=6 912.2	*n*=7 710.8	*n*=6 1141	*n*=6 798.7
Cycle 1, Day 15						
Pre-dose	*n*=3 903.1	*n*=6 560.9	*n*=3 602.5	*n*=4 266.6	*n*=3 819	*n*=2 1099
Post-dose, h						
0.25	*n*=3 963.7	*n*=6 606.8	*n*=2 562.8	*n*=3 496.8	–	–
0.5	*n*=3 1109	*n*=6 881.5	*n*=3 884.4	*n*=3 774	*n*=4 1042	*n*=2 1420
1	*n*=3 1319	*n*=6 1316	*n*=3 1983	*n*=3 1691	*n*=4 1320	*n*=2 1783

1.5	*n*=3 1367	*n*=6 1843	*n*=3 3212	*n*=3 3302	–	–
2	*n*=3 1681	*n*=6 2340	*n*=3 3106	*n*=3 3646	*n*=4 2802	*n*=2 1705
4	*n*=3 1649	*n*=6 1899	*n*=3 2483	*n*=3 2508	*n*=4 2882	*n*=2 2509
6	*n*=3 1657	*n*=6 1637	*n*=2 2130	*n*=3 2023	*n*=4 3291	*n*=2 3401
8	*n*=3 1262	*n*=6 1466	*n*=3 1813	*n*=3 1739	*n*=4 2794	*n*=2 2305
24	*n*=3 919.5	*n*=6 624.5	*n*=3 632.8	*n*=3 459.2	*n*=4 869.7	*n*=2 929.3

Note. Data are G_{mean}, with a limit of quantification = 0.5 ng ml, 20 ng ml. G_{mean} calculated as exp [μ], where μ is the mean of the data on a log scale

Abbreviations: G_{mean} = geometric mean; NC = not calculable

Supplementary Table 4. Concentration and ratio of 4-β-hydroxycholesterol on Day 15 compared with Day 1 in the solid tumour trial

Subject ID	Dose (mg)	Day 1 concentration (ng ml)	Day 15 concentration (ng ml)	Ratio (Day 15/Day 1)
E0001016	700	25.1	85.5	3.41
E0003012	700	15.5	91.9	5.93
E0003014	700	35.7	175	4.90
E0001013	800	27.4	128	4.67
E0002008	800	31.5	110	3.49
G_{mean} (CV%)		26.0 (32.8)	114.1 (29.2)	4.38 (23.9)

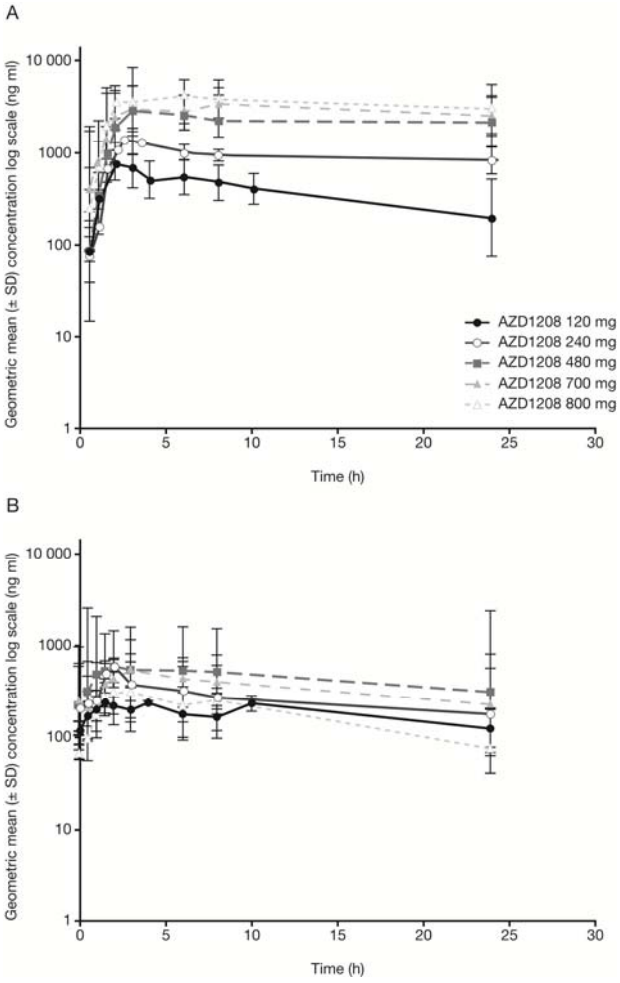
Abbreviations: CV = coefficient of variation; G_{mean} = geometric mean

Supplementary Table 5: Best clinical response following AZD1208 treatment in the AML and solid tumour trials (clinical response analysis set)						
Best response in AML study, *n* (%)	**AZD1208 120 mg *n*=4**	**AZD1208 240 mg *n*=6**	**AZD1208 480 mg *n*=6**	**–**	**AZD1208 700 mg *n*=7**	**AZD1208 900 mg *n*=9**
Modified Cheson criteria						
CR	0	0	0	–	0	0
CRi	0	0	0	–	0	0
Morphologic leukaemia free	0	0	0	–	0	0
Partial remission	0	0	0	–	0	0
Non-response	4 (100)	6 (100)	6 (100)	–	7 (100)	9 (100)
Investigator assessed						
CR	0	0	0	–	0	0
CRi	0	0	0	–	0	0
Morphologic leukaemia free	0	0	0	–	0	0

Partial remission	0	0	0	–	0	0
Non-response	3 (75.0)	3 (50.0)	3 (50.0)	–	1 (14.3)	2 (22.2)
Best response in solid tumour study, *n* (%)	**AZD1208 120 mg *n*=3**	**AZD1208 240 mg *n*=7**	**AZD1208 360 mg *n*=5**	**AZD1208 540 mg *n*=7**	**AZD1208 700 mg *n*=6**	**AZD1208 800 mg *n*=5**
RECIST criteria						
CR	0	0	0	0	0	0
Partial response	0	0	0	0	0	0
Stable disease ≥6 weeks	2 (66.7)	5 (71.4)	1 (20.0)	3 (42.9)	1 (16.7)	1 (20.0)
Progression	1 (33.3)	2 (28.6)	3 (60.0)	3 (42.9)	3 (50.0)	3 (60.0)
Death	0	0	0	0	1 (16.7)	0
Not evaluable	0	0	1 (20.0)	1 (14.3)	2 (33.3)	1 (20.0)
Abbreviations: AML = acute myeloid leukaemia; CR = complete response; Cri = complete response with incomplete recovery of blood counts; RECIST = Response Evaluation Criteria in Solid Tumours						

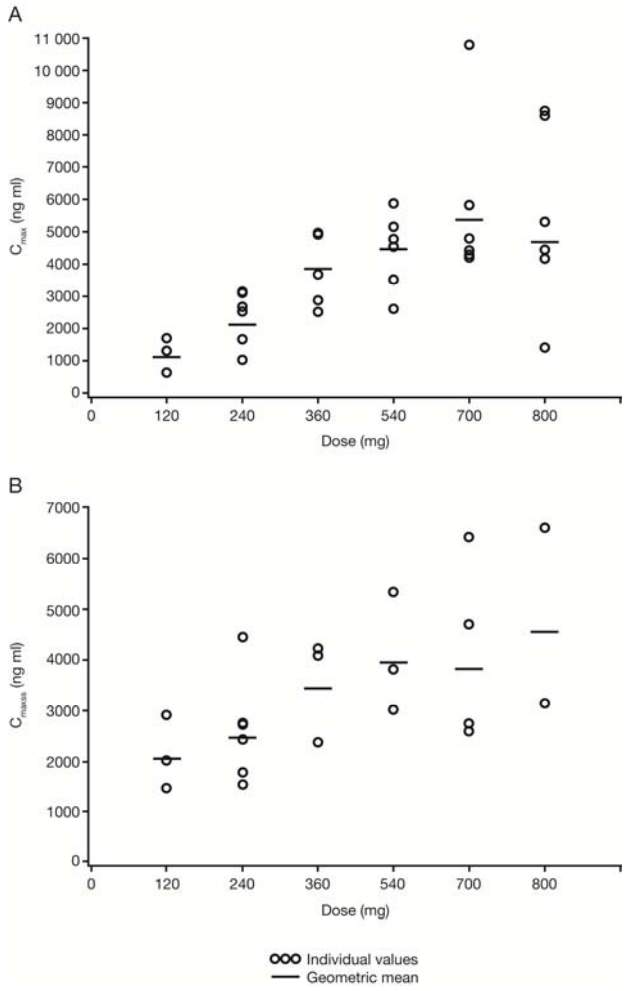
Supplementary Figure 1. AML dose-escalation study geometric mean plasma concentration (± SD) of AZD1208 *vs* time by dose cohort for (A) Cycle 1, Day 1, and (B) Cycle 1, Day 14.

Abbreviations: AML = acute myeloid leukaemia; SD = standard deviation.



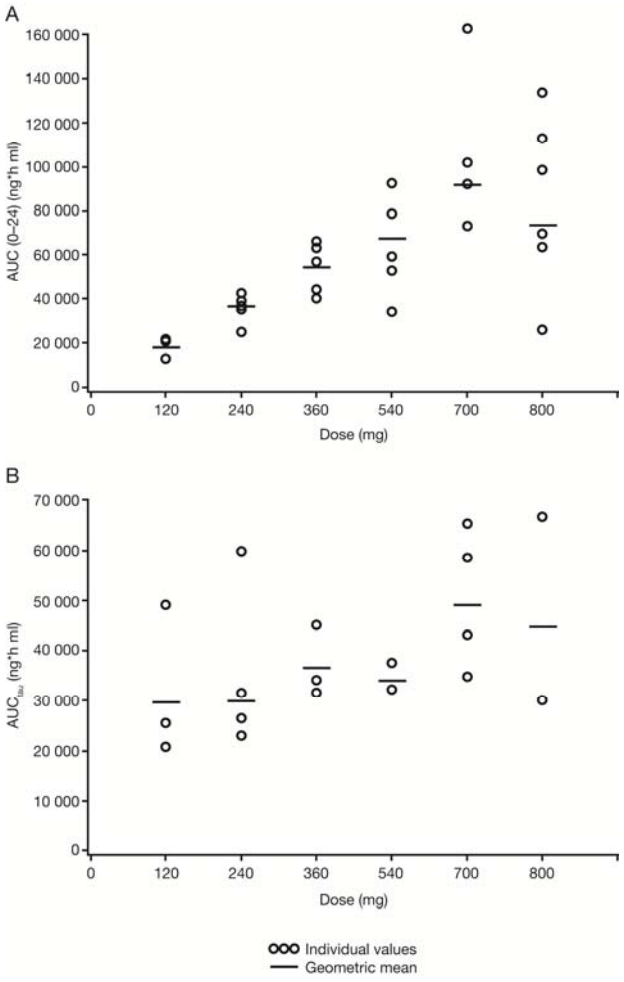
Supplementary Figure 2. Solid tumour dose-escalation study individual and geometric mean values for C_{max} *vs* dose curve for (A) Cycle 0, Day 1, and (B) at steady state on Cycle 1, Day 15.

Abbreviation: C_{max} = maximum plasma concentration.



Supplementary Figure 3. Solid tumour dose-escalation study individual and geometric mean values for AUC for (A) 0–24 h on Cycle 0, Day 1, and (B) 0 h to the end of treatment on Cycle 1, Day 15.

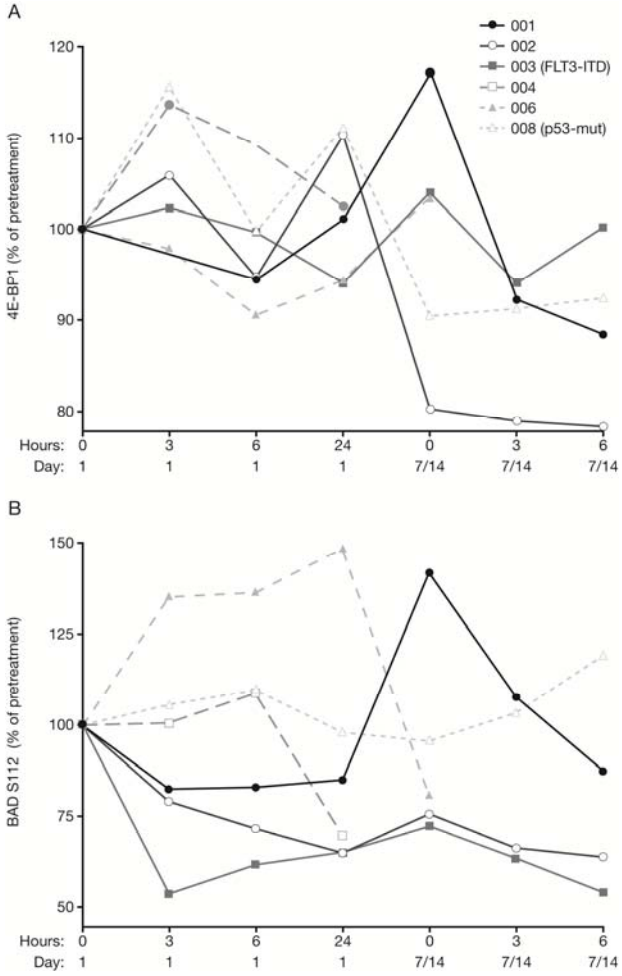
Abbreviation: AUC = area under plasma concentration–time curve.



Supplementary Figure 4. Measurement of cellular protein level changes by RPPA in AML blasts during therapy with AZD1208. Graphical representation of relative, normalised linear protein levels determined by RPPA of (A) 4E-BP1 S65, and (B) BAD S112.

Cellular proteins were extracted from AML blasts from patients (*n*=6) isolated during therapy with AZD1208, and were analysed by RPPA. For all patients, samples were collected on Day 1. For the first two patients, the second set of samples were collected on Day 7 (dashed lines) and for the last four patients the second set of samples were collected on Day 14 (solid lines). One patient had an FLT3-ITD mutation and one patient had p53 mutation.

Abbreviations: AML = acute myeloid leukaemia; FLT3 = FMS-like tyrosine kinase 3; ITD = internal tandem duplication; mut = mutation; RPPA = reverse phase protein array.


A
001
002
003 (FLT3-ITD)
004
006
008 (p53-mut)
4E-BP1 (% of pretreatment)
120
110
100
90
80
Hours: 0 3 6 24 0 3 6
Day: 1 1 1 1 7/14 7/14 7/14
B
BAD S112 (% of pretreatment)
150
125
100
75
50
Hours: 0 3 6 24 0 3 6
Day: 1 1 1 1 7/14 7/14 7/14

Supplementary Figure 5. (A) RPPA heatmap of modulated protein levels in AML blasts during therapy with AZD1208. Cellular proteins were extracted from AML blasts from patients (*n*=6) isolated during therapy with AZD1208 and were analysed by RPPA. Graphical representation of changes in phosphorylated protein levels are shown in (B) 4E-BP1 T37/46, (C) PRAS40 T246 and (D) mTOR S2448.

The heatmap was generated using unsupervised clustering analysis.

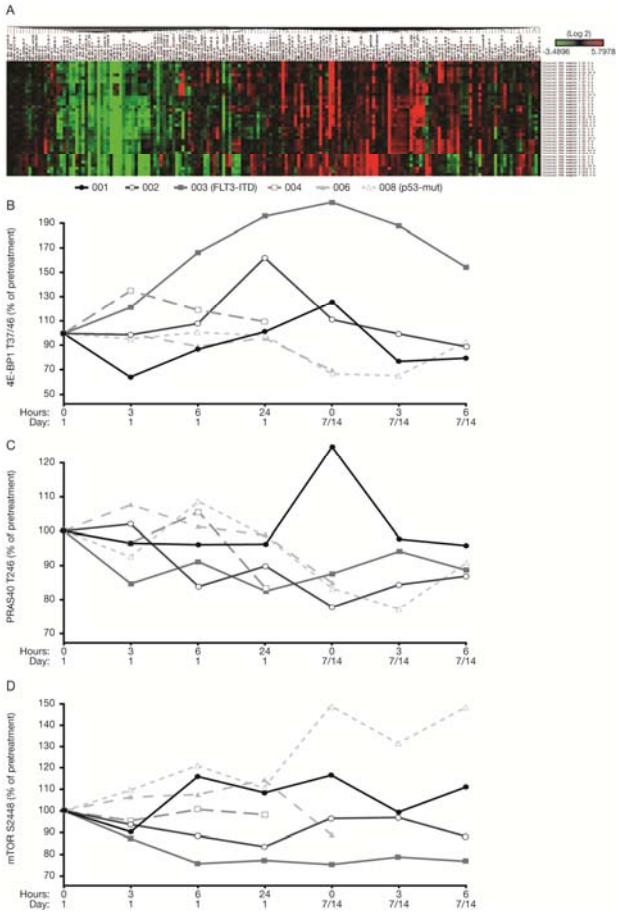
For all patients, samples were collected on Day 1.

For the first two patients, the second set of samples were collected on

Day 7 (dashed lines) and for the last four patients the second set of samples were collected on Day 14 (solid lines).

One patient had an FLT3-ITD mutation and one patient had p53 mutation.

Abbreviations: AML = acute myeloid leukaemia; FLT3 = FMS-like tyrosine kinase 3; ITD = internal tandem duplication; RPPA = reverse phase protein array.


A
(Log 2)
-3.4896
5.7978
001
002
003 (FLT3-ITD)
004
006
008 (p53-mut)
B
4E-BP1 T37/46 (% of pretreatment)
C
PRAS40 T246 (% of pretreatment)
D
mTOR S2448 (% of pretreatment)
Hours:
Day: