Supplementary Tables

Supplementary Table 1. Response to gilteritinib in subjects treated on the CHRYSALIS study

Variable	**Number (%)** n=32
Response	
CR/CRi	13 (40.6)
PR	12 (37.5)
No response	7 (21.9)
Median duration of gilteritinib, wks (range)	20.0 (3.7-76.7)
Reason for gilteritinib discontinuation	
Relapse/Progression	22 (68.8)
Toxicity	3 (9.4)
Allogeneic HSCT	4 (12.5)
Death/Hospice	3 (9.4)

Abbreviations: CR, complete remission; CRi, CR with incomplete count recovery; PR, partial remission; wks, weeks; HSCT, hematopoietic stem cell transplantation.

Supplementary Table 2. New mutations identified after gilteritinib treatment

Pt.	Disease-associated mutations (VAF %)	VUS (VAF %)
1	None	None
2	*NRAS* G13D (6%)	None
3	*IDH2* R140Q (40.6%), *NRAS* G12S (19.2%), *NRAS* G12D (12.2%)	*NRAS* R68S (11.0%)
4	None	*FLT3* M837K (10.1%)
5	*CEBPA* G151Rfs*32	None
6	None	None
7	*FLT3* F691L (15.0%), *RUNX1* T270fs (48.0%)	None
8	None	None
9	None	None
10	None	None
11	None	None
12	*NRAS* G13R (34.8%)	*TET2* G1275A (15.5%)
13	*FLT3* F691L (5.8%)	None
15*	None	None
16	*CBL* Q367_E373delinsRLK (5.1%), *NRAS* G12D (29.9%)	None
17	*FLT3* F691L	None
18	TBL1XR1 A142Ifs*4 (40.1%)	None
19	*NRAS* Q61K (14.0%)	None
20	None	None
21	*NRAS* G13R (9.5%)	*RUNX1* G199R (9.6%)
22	None	None
23*	*WT1* A382Vfs*4 (7%), *WT1* p.? (5%)	None
24	*FLT3* F691L (48.0%)	None
25	*NRAS* Q61L (8%), *PTPN11* G60R (15.0%), *PTPN11* G503A (15.0%)	None
26	*KRAS* G12S (13.0%), *NRAS* G12C (5.0%)	None
27	None	*PTPN11* F71L (9%)
28	*CBL* C404Y (31%), *BRAF* G469A (16%)	None
29	*NRAS* Q61R (44.0%), *WT1* L378Pfs*6 (40%)	None
30	*NRAS* Q61K (45.0%)	None
31	None	None
32	None	*FLT3* C35S (10.0%)
33	*KRAS* G12V (37.0%), *PTPN11* S502L (5.0%)	*TET2* (46.0%)
34	*KRAS* G12A (6.0%), *NRAS* Q61R (6.0%), *PTPN11* G503A (66.0%)	None
35	None	None
36	None	None
37	None	None

38	*NRAS* G13C (5%), *NRAS* G13R (39%)	None
39	*NRAS* Q61H (30.0%)	None
40	None	None
41	*FLT3* F691L (10.0%)	None

*Subjects 15 and 23 had new *BCR-ABL1* fusions detected following gilteritinib treatment.
Abbreviations: VAF, variant allele frequency; VUS, variant of uncertain significance

Supplementary Table 3. Cytogenetic evolution on gilteritinib

Pt. ID	Baseline karyotype	Post-gilteritinib karyotype	Timing of cytogenetic evolution*
1	46,XY[20]	46,XY,**add(8)(p21)**[18]/46,XY[6]	Progression
5	46,XX,t(3;10)(q21;p13)[14]//46,XY[1]	47,XX,t(3;10)(q21;p13),+**21**[16]// 46,XY[4]	Progression
6	46,XY,t(15;17)(q24;q21), add(17)(p12)[20]	46,XY,t(15;17)(q24;q21),add(17)(p12)[14] /46,idem,**del(1)(q32)**,**der(1)add(1)(q21) t(1;3)(p22;p?13),der(3)t(1;3)**[4]/46,idem, **t(12;13)(p13;q12)**[cp2]	Progression
8	46,XY[20]	46,XY,**del(20)(q12)**[5]/46,XY,**t(6;17)(p12 ;q12)**[4]/46,XY[12]	Progression
12	46,XX,t(3;8)(q26;q24)[20]	46,XX,t(3;8)(q26;q24)[14]/46,sl,**der(1) t(1;9)(q21;q22),der(9)t(1;9)(q21;q13)**[6]	During response
13	46,XX,del(11)(p?12p15)[9]/46,XX[11]	46,XX,**t(7;11)(q32;q13)**[3]/46,XX[5]	During response
15†	46,XY,del(11)(q13q23)[3]/46,XY[17]	46,XY,**t(9;22)(q34;q11.2)**[3]/46,idem, **t(1;3)(p36.1;q21)**[15]/46,XY[2]	Progression
19	47,XX,add(3)(q?12),+add(8)(p12), t(11;15)(q23;q15)[15]/46,XX[6]	48~49,XX,add(3)(q12),**-8**,t(11;15) (q23;q15),+**3,+mar**[15]/49~51,idem,+**4, +19,+21**[cp5]	Progression
20	46,XY,t(3;7)(p21;p15)[7]/46,XY[7]// 46,XX[6]	46,XY,**inv(2)(p13q32)**,t(3;7)(p21;p15)[20] //	During response
23†	46,XY,t(2;15)(q11.2;q11.2)[5]/46,idem,a dd(22)(q13)[11]/46,XY[3]//46,XX[1]	46,XY,**t(9;22)(q34;q11.2)**[20]//	During response
25	46,XX,del(20)(q11.2q13.3)[1]/47,idem, del(3)(q?12q?21),+8[19]	47,XX,+8,del(20)(q11.2q13.3)[2]/47,idem, del(3)(q12q21)[8]/48,idem,del(3)(q12q21) ,+**11**[7]/46,XX,del(20)(q11.2q13.3), **del(21)(q22.1q22.3)**[3]	During response
26	46,XY,del(5)(q22q3132)[17]/46,XY[3]	46,XY,del(5)(q22q31 32)[10] /46,idem,**t(12;13)(q15;q14)**[10]	Progression
29	46,XX[16]	46,XX,**t(3;16)(q12;q?23)**[10]/46,XX,**t(3;7)(q21;p13)**[7]/46,XX[3]	Progression
36	46,XX,t(12;17)(p13.2;p13.3){20}	46,XX,t(12;17)(p13.2;p13.3)[3]/ 46,XX,**add(6)(p25)**,t(12;17)(p13.2;p13.3) [2]/46,XX,**add(6)(q21)**,t(12;17) (p13.2;p13.3)[6]/46,XX,t(12;17) (p13.2;p13.3),**add(19)(p13.3)**[9]	Unknown
38	46,XX[20]	47XX,+**4**[17],46,XX[3]	Unknown
39	46,XY[21]	46,XY,**t(3;17)(p21;q25)**[13]/46,XY[7]	Unknown

*For patients with serial cytogenetic data available, results were analyzed to determine whether the new karyotypic abnormalities were initially detected while the patient was otherwise clinically and morphologically responding to gilteritinib or whether the karyotypic abnormalities were first noted at the time of AML progression.
†Subjects 15 and 23 developed new *BCR-ABL1* fusions (t(9;22)) on gilteritinib.

Supplementary Table 4. Clinical responses to gilteritinib in selected patients

Pt. ID	Day	Gilteritinib dose (mg/day)	WBC ($x10^9$/L)	ANC (cells/uL)	PB blasts (%)	Hgb (g/dL)	Plts ($x10^9$/L)	BM blasts (%)	Clinical Response
3	1	200	1.8	360	0	7.7	37	90	N/A
	88	200	1.9	1090	0	11.7	149	0	CR
	173	200	2.1	950	0	11.5	149	1	CRi
	258	200	6.2	4020	5	10.4	76	10	Relapse
7	1	80	1.7	30	43	8.3	54	90	N/A
	29	80	1.0	40	20	9.2	24	74	Stable disease
	57	80	1.0	0	24	9.9	14	90	Progression
12	1	120	94.3	1850	95	9.0	43	75	N/A
	31	120	6.4	2230	46	8.9	69	30	<PR
	64	120	4.9	3680	6	10.1	65	15	PR
	120	120	22.3	1474	14	9.5	30	10	PR
	173	120	65.7	8290	72	8.7	160	90	Relapse
21	1	120	9.6	670	61	8.2	86	37	N/A
	29	120	3.6	650	1	10.4	14	23	<PR
	57	200	4.5	620	1	9.0	25	8	PR
	113	200	6.3	1600	17	7.9	108	9	PR
	169	200	12.0	2520	15	10.1	90	15	Progression
30	1	200	0.3	0	Present	10.6	41	90	N/A
	60	Held	2.6	280	<5%	11.5	39	>90	N/A*
	207	200	2.9	2380	Rare	8.8	32	<5†	CRp
	253	200	103.6	48690	35	8,7	59	70	Relapse
33	1	300	3.4	1810	0	10.9	176	40	N/A
	29	300	3.4	1000	0	13.2	58	1	CRi
	43	300	30.2	2720	<5%	12.9	85	ND	Progression

*Gilteritinib had been held for 22 days prior to this response assessment due to liver function test abnormalities.
†Bone marrow biopsy was performed on day 168 (blood tests from day 207).
Abbreviations: Pt., patient; ID, identification; mg, milligrams, WBC, white blood cell count; ANC, absolute neutrophil count; PB, peripheral blood; Hgb, hemoglobin; Plts, platelet count; BM, bone marrow; N/A, not applicable; CR, complete remission; CRp, complete remission with incomplete platelet recovery; CRi, complete remission with incomplete hematological recovery; PR, partial remission; Unk, unknown; uL, microliter; ND, marrow evaluation not done.

Supplementary Table 5. Total number of cells sequenced at each timepoint for serial single-cell sequencing analyses.

Patient ID	**Sample**	**Timepoint** (day)	**Total number of cells sequenced**
12	1	Baseline	8,462
	2	31	6,304
	3	185	3,378
30	1	Baseline	15,485
	2	91	11,468
	3	239	7,671
	4	285	4,201
21	1	Baseline	13,709
	2	28	7,725
	3	112	11,673
	4	204	6,520
25	1	Baseline	6,048
	2	28	9,285
	3	56	8,990

Supplementary Table 6. Genes included on the University of Pennsylvania Center for Personalized Diagnostics targeted next-generation sequencing panel

Version 1 (33-gene panel):

ASXL1	*ATM*	*BRAF*	*CBL*	*CDKN2A*	*DDX3X*	*DNMT3A*
ETV6	*EZH2*	*FBXW7*	*FLT3*	*GNAS*	*IDH1*	*IDH2*
JAK2	*KIT*	*KLHL6*	*KRAS*	*MAPK1*	*MYD88*	*NOTCH1*
NPM1	*NRAS*	*PHF6*	*PTEN*	*PTPN11*	*RUNX1*	*SF3B1*
TET2	*TP53*	*WT1*	*XPO1*	*ZMYM3*		

Version 2 (68-gene panel) additionally includes:

ABL1	*BCOR*	*BCORL1*	*BIRC3*	*CALR*	*CEBPA*	*CSF1R*
CSF3R	*FAM5C*	*GATA2*	*HNRNPK*	*IL7R*	*MAP2K1*	*MIR142*
MPL	*MYC*	*MYCN*	*NF1*	*NOTCH2*	*PDGFRA*	*POT1*
PRPF40B	*RAD21*	*RIT1*	*SETBP1*	*SF1*	*SF3A1*	*SMC1A*
SRSF2	*STAG2*	*TBL1XR1*	*TPMT*	*U2AF1*	*U2AF2*	*ZRSR2*