

Supplementary Materials for

CHK1 as a therapeutic target to bypass chemoresistance in AML

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Published 13 September 2016, *Sci. Signal.* **9**, ra90 (2016)
DOI: 10.1126/scisignal.aac9704

The PDF file includes:

- Fig. S1. Kaplan-Meier survivor functions according to *BAALC*, *ERG*, or *MNI* expression.
- Fig. S2. Analysis of relapse and death.
- Fig. S3. Overall survival, relapse-free survival, and relapse incidence according to *CHEK1* expression in allografted patients.
- Fig. S4. Kaplan-Meier overall survivor functions according to *CHEK1* expression in the favorable-risk group.
- Table S1. Clinical characteristics of the cohort.
- Table S2. Overall survival according to *BAALC*, *ERG*, or *MNI* expression.
- Legend for table S3
- Table S4. Quantification method to determine CHK1 abundance in patient samples.

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/9/445/ra90/DC1)

Table S3 (Microsoft Excel format). List of the genes tested for the analysis.

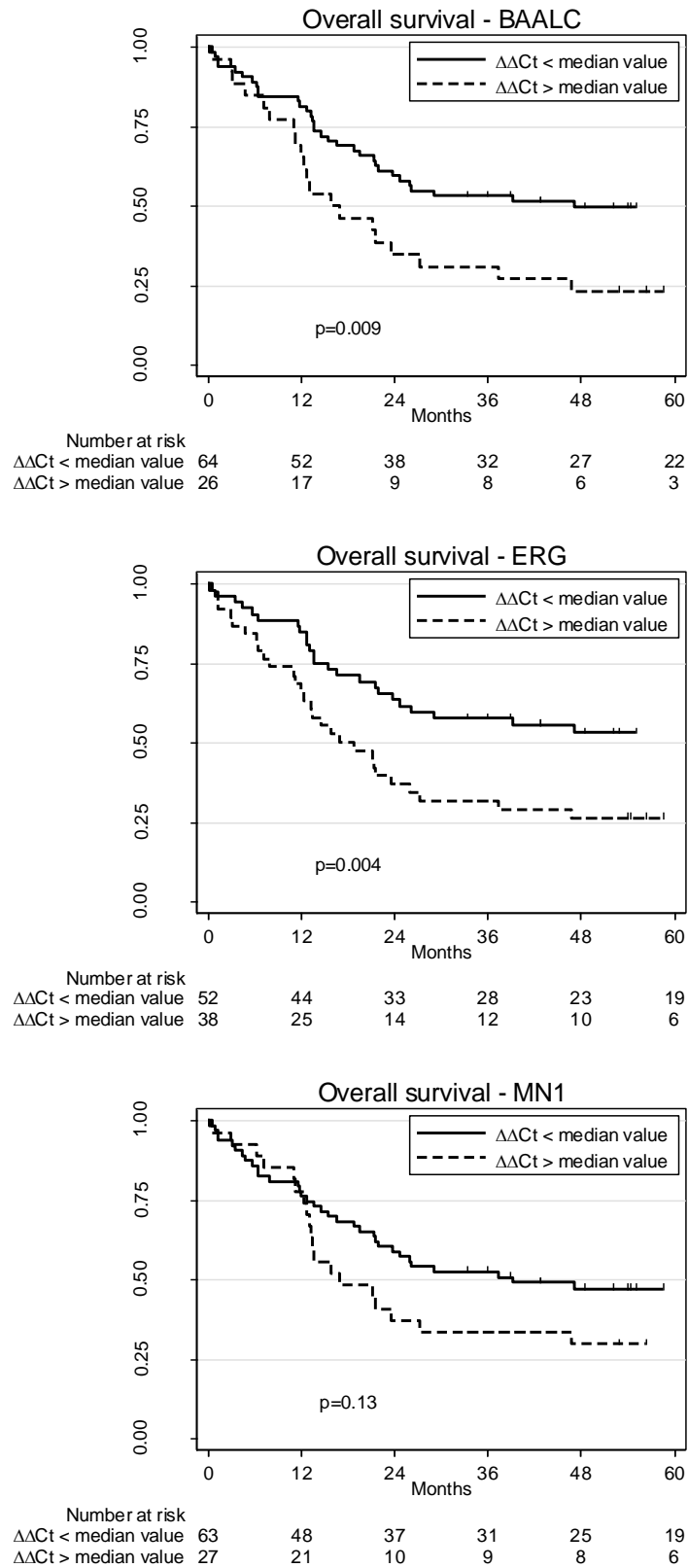


Figure S1: Kaplan-Meier survivor functions according to BAALC, ERG, or MN1 expression. Data are from 90 patients.

Figure S2A

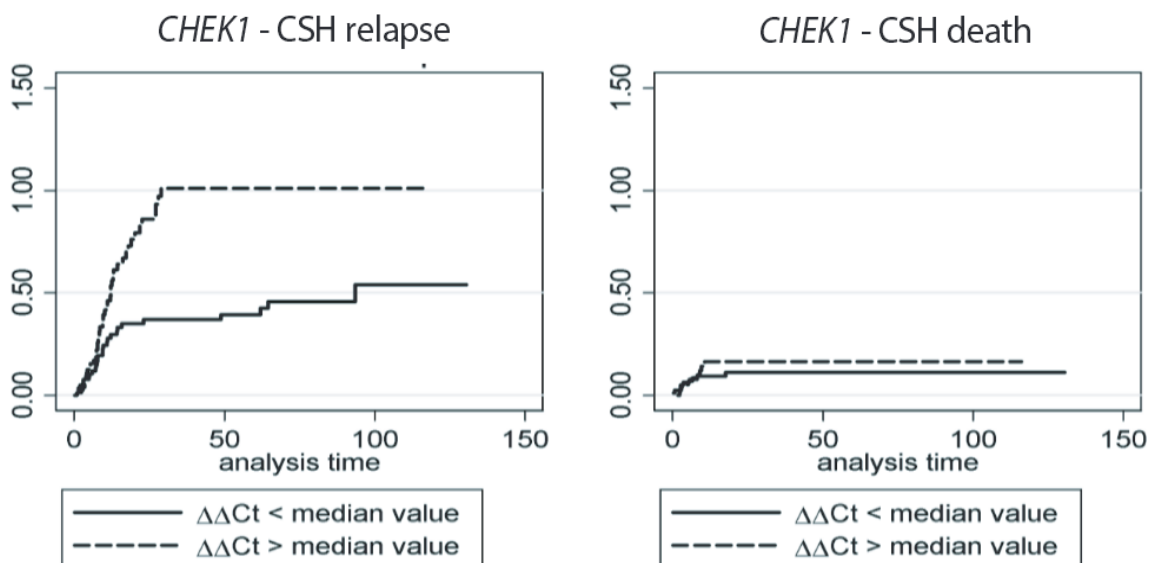


Figure S2B

	Cox : cause specific hazard ratio (HR)					
	relapse			death of other cause		
	HR	(95% CI)	p	HR	(95% CI)	p
<i>CHEK1</i> unadjusted	2.11	(1.31 ; 3.39)	0.002	1.39	(0.55 ; 3.52)	0.49
<i>CHEK1</i> adjusted*	2.32	(1.41 ; 3.80)	0.001	1.26	(0.47 ; 3.34)	0.65

	Fine-Gray : subdistribution hazard ratio (HR)					
	relapse			death of other cause		
	HR	(95% CI)	p	HR	(95% CI)	p
<i>CHEK1</i> unadjusted	1.97	(1.23 ; 3.18)	0.005	1.31	(0.52 ; 3.33)	0.56
<i>CHEK1</i> adjusted*	2.19	(1.32 ; 3.65)	0.003	1.20	(0.42 ; 3.40)	0.73

* adjusted for year of diagnosis, age, sex, white blood cells count and cytogenetic risk group

Figure S2: Analysis of relapse and death. (A) Cumulative cause-specific hazards of relapse (CSH) (left) and death (right) according to *CHEK1* expression. **(B)** Estimated cause specific hazard and subdistribution hazard ratios for relapse and death of other cause using unadjusted and adjusted regression models.

Figure S3A.

Overall	<i>CHEK1</i> expression	N	Events	Survival (months)		p (Log-rank test)
				1st quantile	Median	
Survival						
in the sub-group with allograft (n=56*)	< median	24	7	26.4	-	0.0005
	> median	32	24	6.0	19.1	

* one unknown value

Figure S3B

Relapse free	<i>CHEK1</i> expression	N	Events	Survival (months)		p (Log-rank test)
				1st quantile	Median	
Survival						
in the sub-group with allograft (n=49*)	< median	22	5	-	-	0.0004
	> median	27	20	5.7	9.4	

* one unknown value

Figure S3C

	Cox : cause specific hazard ratio (HR)					
	relapse			death of other cause		
	HR	(95% CI)	p	HR	(95% CI)	p
<i>CHEK1</i> unadjusted	6.30	(1.79 ; 22.11)	0.004	3.14	(0.63 ; 15.68)	0.16

	Fine-Gray : subdistribution hazard ratio (HR)					
	relapse			death of other cause		
	HR	(95% CI)	p	HR	(95% CI)	p
<i>CHEK1</i> unadjusted	4.84	(1.37 ; 17.07)	0.01	2.67	(0.55 ; 12.90)	0.22

* one unknown value

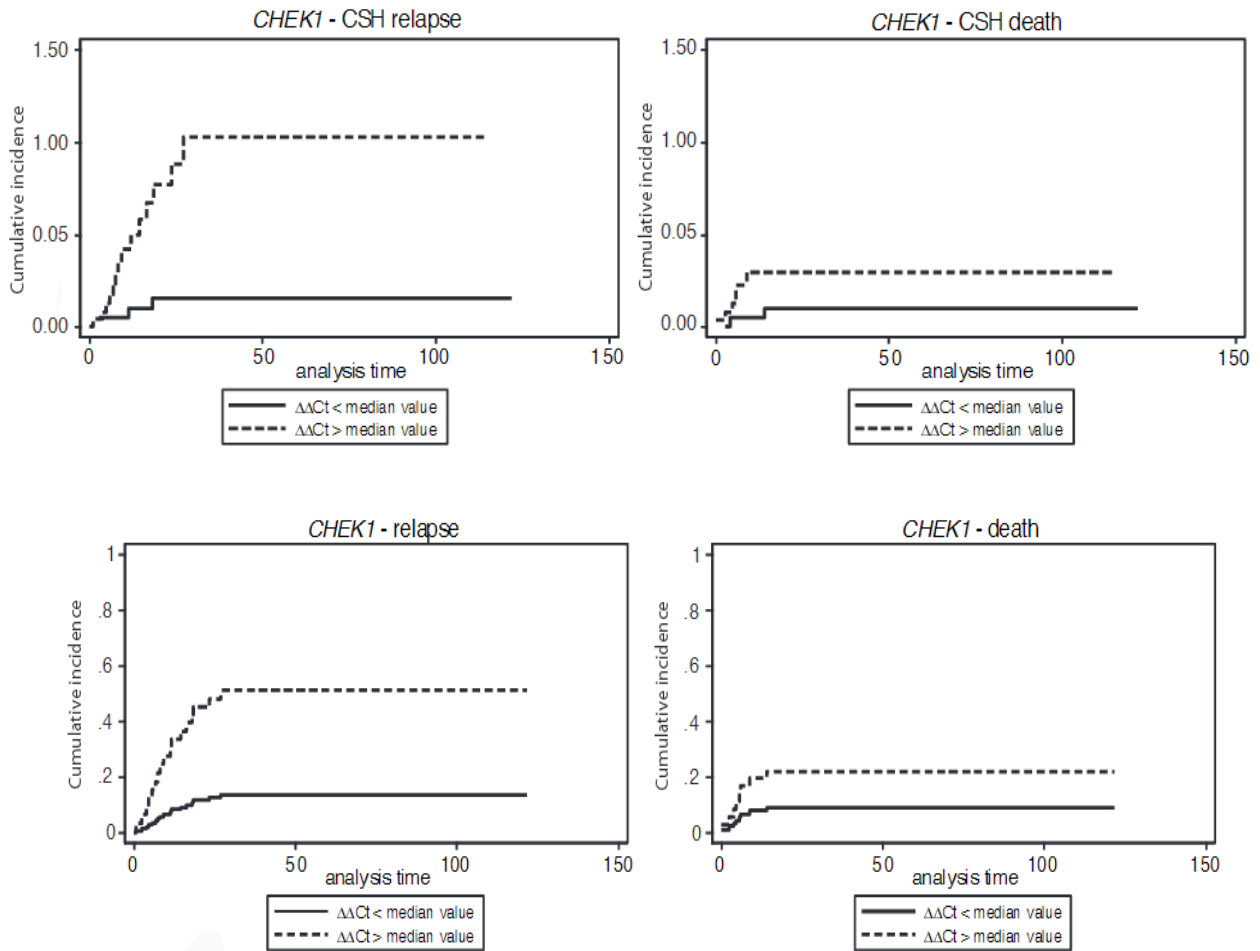


Figure S3: Overall survival, relapse-free survival, and relapse incidence according to *CHEK1* expression in allografted patients. (A) Overall survival in patients who were allografted in first CR (n=50). (B) Relapse-free survival in patients who were allografted in first CR (n=50). (C) Cumulative cause-specific hazards of relapse (CSH) and death (upper panels) and cumulative incidence of relapse and death (lower panels) in patients who were allografted in first CR (n=50).

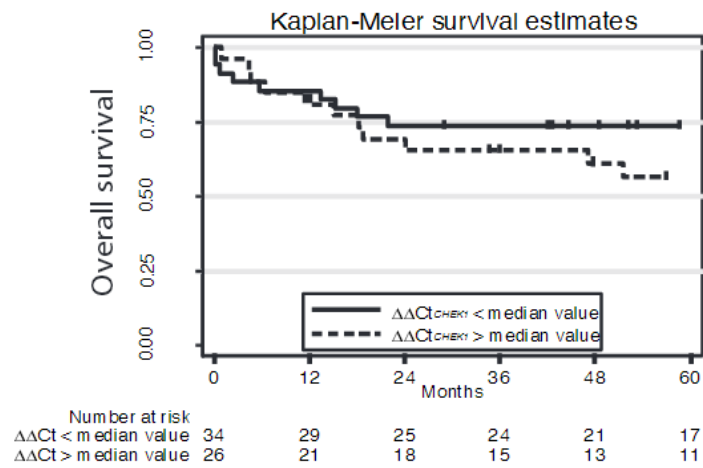


Figure S4: Kaplan-Meier overall survivor functions according to *CHEK1* expression in the favorable-risk group. The favorable-risk group (n=60) including patients with core binding factor-AML and patients with *NPM1* mutation without *FLT3* mutation (log-rank test p-value=0.36).

Table S1: Clinical characteristics of the cohort. The clinical characteristics of the 513 AML patients (18-65 years) treated by intensive chemotherapy (315 not tested and 198 tested for *CHEK1* expression) are presented.

	Not tested cohort (n=315)		Study population (n=198)	
age (median, [min, max])	54.7	[15.4, 65.9]	48.5	[18.5, 65.2]
White blood Cells in G/L (median, [min, max])	5.4	[0.3, 327]	30.3	[0.7, 356]
Secondary AML (n, %)				
No	225	71.4%	169	85.4%
Yes	89	28.3%	28	14.1%
unknown	1	0.3%	1	0.5%
Cytogenetic risk group (n, %)				
Favorable	28	8.9%	31	4.0%
Intermediate	191	60.6%	129	65.2%
Adverse	85	27.0%	38	19.2%
Unknown	11	3.5%	0	0%
Treatment response (n, %)				
Complete response	245	77.8%	162	81.8%
Failure	39	12.4%	26	13.1%
Early death	31	9.8%	10	5.1%
Median Overall survival (months)	22.4		26.1	
Relapse Free survival (months)	24.6		21.9	

Table S2: Overall survival according to BAALC, ERG, or MN1 expression. The Kaplan-Meier estimates and hazard ratios in Cox multivariate models were adjusted for age, gender, white blood cells, and year of diagnosis. Overall survival was estimated according to BAALC, ERG, or MN1 expression in 90 patients with a normal karyotype. The 90 patients were divided into a high and a low expression group, according to the median expression value. Overall survival time and hazard ratios are detailed in Table S1, the estimated Kaplan-Meier survivor functions are showed in Figure S1.

	$\Delta\Delta$ Ct	N total	n deaths	Kaplan Meier overall survival time			multivariate Cox model		
				1st quartile	median	p*	HR	(95% CI)	p
BAALC	<-0.09	64	33	13.6	47.1	0.009	1.00		
	>-0.09	26	21	11.0	15.8		2.11	(1.19; 3.74)	0.01
ERG	<0.47	52	25	13.6	98.4		1.00		
	>0.47	38	29	8.0	16.9	0.004	2.30	(1.32; 4.00)	0.003
MN1	<-0.23	63	34	12.6	39.2	0.09	1.00		
	>-0.23	27	20	12.4	16.9		1.63	(0.86; 3.07)	0.13

* log rank test

Table S3: List of the genes tested for the analysis. Presentation of all the genes tested and the statistical analysis for each of them for overall survival, event-free survival, and relapse-free survival. Data are provided in an Excel file with a separate worksheet for each survival analysis.

Table S4: Quantification method to determine CHK1 abundance in patient samples. Within a cohort of 37 AML patient samples cohort, CHK1 abundance was determined by immunoblot in 30 samples and/or by immunofluorescence in 22 samples. **(A)** Presentation of the quantification method used for each patient of the cohort and the classification as “Low Chk1” or “High Chk1” abundance. **(B)** Immunoblot quantifications were performed from the ratio between CHK1 values in AML samples (2nd column) and CHK1 value in the KG1a cell line used as a between-gels control. These ratios are presented in the 3rd column. Actin protein values were also quantified (4th column) then normalized with actin protein value of the KG1a cell line (5th column). Finally, CHK1 abundance (6th column) in patient samples was determined by a ratio between values of the 3rd column and the 5th column. AML patient cells are considered “high CHK1” abundance if CHK1 abundance is 1.2 fold higher than the median (values are presented in the 7th column and classification “Low CHK1” or “High CHK1” abundance are presented in the 8th column). **(C)** Mean, maximal, minimal and median intensity of immunofluorescence per cell was quantified for 22 patients with ImageJ software 1.48V. At least 100 cells were scored for each patient sample. Average values of mean, maximal, minimal and median intensity obtained for each sample analyzed are presented in the 2nd, 3rd, 4th and 5th columns respectively. Results “Low CHK1” or “High CHK1” abundance are presented in the 6th column. The three subsections of the table are shown on the next pages.

Table S4A: CHK1 abundance in each patient sample of the cohort.

	Quantification by immunoblot	Quantification by immunofluorescence	CHK1 abundance
#1	X		Low CHK1
#4	X	X	Low CHK1
#8	X	X	High CHK1
#10	X		Low CHK1
#11	X		Low CHK1
#15		X	Low CHK1
#16	X	X	High CHK1
#17	X	X	High CHK1
#18		X	Low CHK1
#19	X		Low CHK1
#20		X	High CHK1
#21	X	X	Low CHK1
#22	X		Low CHK1
#23	X	X	Low CHK1
#24	X	X	High CHK1
#25	X	X	High CHK1
#26		X	High CHK1
#27		X	High CHK1
#28		X	High CHK1
#29	X	X	Low CHK1
#30		X	Low CHK1
#31	X		Low CHK1
#33	X	X	Low CHK1
#34	X	X	Low CHK1
#36	X	X	Low CHK1
#38	X	X	High CHK1
#39	X		Low CHK1
#40	X		Low CHK1
#41	X		Low CHK1
#42	X		High CHK1
#44	X		High CHK1
#45	X	X	High CHK1
#46	X		High CHK1
#47	X		High CHK1
#48	X	X	Low CHK1
#50	X		Low CHK1
#51	X		High CHK1

Table S4B. Immunoblot quantification.

	CHK1 quantification	CHK1 / CTL	Actin quantification	Actin / CTL	CHK1/CTL / Actin/CTL	Value / 1.2*Mediane	CHK1 abundance
#1	8082	0.158844339622642	53159	1.37910548435635	0.115179253091562	0.20366875550401	Low Chk1
#4	10479	0.205955188679245	37829	0.981398848129508	0.209858804166914	0.371088371638056	Low Chk1
#10	25450	0.162225905150433	8728527	0.348569815900118	0.465404340107633	0.683802009667412	Low Chk1
#11	32943	0.209988526262111	22186320	0.886000751089055	0.237007165066166	0.348226180573091	Low Chk1
#19	23210	0.387317480183563	110471	0.641687529406298	0.603592032623599	0.88683626119989	Low Chk1
#21	440	0.00491515767602408	7570	0.128235533270091	0.0383291397531115	0.0677765134216468	Low Chk1
#22	4206	0.0469844390576302	13347	0.226097709716764	0.20780590443171	0.367458277475948	Low Chk1
#23	11370	0.127012142673622	30975	0.52471540859195	0.242059105934117	0.428027405463587	Low Chk1
#29	5331640	0.225561784918324	1017701	0.597278824529197	0.377649057115196	0.592305445295918	Low Chk1
#31	4280990	0.181112705587304	1074403	0.63055667716809	0.287226687378372	0.440170713560115	Low Chk1
#33	0.00	0.00	1254098	0.736017925976703	0.00	0.00	Low Chk1
#34	4432443	0.187520116629917	929149	0.545308516482227	0.343878943684219	0.541131712310985	Low Chk1
#36	23479	0.391806424697539	143129	0.831386467003956	0.471268706248588	0.692418313829258	Low Chk1
#39	0.00	0.00	241181	1.40093635460655	0.00	0.00	Low Chk1
#40	0.00	0.00	3250369	0.359231936580768	0.00	0.00	Low Chk1
#41	1676510	0.0512427163337515	7228457	0.268658511147127	0.190735503278693	0.8333333333333333	Low Chk1
#48	0.00	0.00	8024990	0.886924745695426	0.00	0.00	Low Chk1
#50	0.00	0.00	26168	0.2434413723812	0.00	0.00	Low Chk1
#8	121569	2.38932783018868	67204	1.74347532817932	1.37043971404162	2.42331620982335	High CHK1
#16	38836	0.147168495725459	73922	0.231265204260578	0.636362466182492	2.78030070981189	High CHK1
#17	127198	0.810798062213157	29600996	1.18210251582886	0.685894879129537	1.00776090025481	High CHK1
#24	73984	0.82646142159765	40096	0.228415873554502	3.61823111825216	6.39803271162459	High CHK1
#25	34204	0.382086484433472	26315	0.445775172787641	0.857128229111789	1.51563962297776	High CHK1
#38	111933	1.86788485607009	167527	0.973105943992983	1.9195082175796	2.82026501181852	High CHK1
#42	3094933	0.0945969745429293	6072137	0.225681813684633	0.419160822037343	1.83133543061849	High CHK1
#44	4584855	0.140136607712678	4958600	0.184295222808152	0.760391970976688	3.32219206661298	High CHK1
#45	1328417	0.0406032147162457	1816906	0.0675285556591512	0.601274739551509	2.62700060736708	High CHK1
#46	4353445	0.133063535087526	1813913	0.0674173154700122	1.97372936255099	8.62332623197704	High CHK1
#47	7967400	0.243524475319283	2574076	0.0956701306709788	2.54545983800099	11.1212464129873	High CHK1
#51	25072	0.095010004244248	51867	0.162266068956243	0.585519849315172	2.55816667256594	High CHK1

Table S4C : Immunofluorescence quantification.

	Mean	Min	Max	Median	CHK1 abundance
#15	4786.54874561404	2144.76315789474	9560.01754385965	4760.45614035088	Low CHK1
#21	8646.9775	3523.5	16048.5	8324.5	Low CHK1
#20	9264.98834313726	4569.94117647059	13379.1470588235	9443.87254901961	High CHK1
#26	8923.73492380952	4259.28571428571	13632.0380952381	9101.78095238095	High CHK1
#4	2659.61175177305	1348.17021276596	4936.10638297872	2657.14184397163	Low CHK1
#18	3318.63377575757	1633.62424242424	5582.7696969697	3347.96363636364	Low CHK1
#23	3532.74628828829	1832.52252252252	6119.06306306306	3505.64864864865	Low CHK1
#24	3889.38671323529	1951.52941176471	6718.875	3876.63970588235	High CHK1
#48	106.429865168539	57.5393258426966	396.134831460674	101	Low CHK1
#16	159.566078651685	84.9775280898876	378.067415730337	155.820224719101	High CHK1
#45	190.065333333333	93.4	563.133333333333	183.016666666667	High CHK1
#29	6906.09349230769	3940.24615384615	10876.2923076923	6934.33076923077	Low CHK1
#30	5538.66894202899	3166.84782608696	9313.49275362319	5522.80434782609	Low CHK1
#33	4704.025	2227.79245283019	9534.13836477987	4671.13836477987	Low CHK1
#34	4622.80285620916	2540.6339869281	8535.13071895425	4612.93464052288	Low CHK1
#36	3730.49889655172	1726	7047.24137931035	3714.10344827586	Low CHK1
#17	7316.1787	4782.87	10423.44	7301.34	High CHK1
#25	8019.10213861386	4477.36633663366	11823.0594059406	7968.50495049505	High CHK1
#26	10009.9677962963	6448.7962962963	13869.0185185185	10030.8518518519	High CHK1
#27	9396.43024786325	5929.98290598291	13568.8717948718	9391.58974358974	High CHK1
#28	9403.12948039216	5507.54901960784	13673.2843137255	9284.1568627451	High CHK1
#38	10046.6526	5712.11428571429	13406.0142857143	10232.7428571429	High CHK1
#4	2659.61175177305	1348.17021276596	4936.10638297872	2657.14184397163	Low CHK1
#8	3716.84730935252	1686.05755395683	6210.34532374101	3741.65467625899	High CHK1
#24	3889.38671323529	1951.52941176471	6718.875	3876.63970588235	High CHK1