Supplemental Information

Multigene measurable residual disease assessment improves AML relapse risk stratification in autologous hematopoietic cell transplantation

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SUPPLEMENTAL METHODS

RQ-PCR Information

Previously validated, specific RQ-PCR assays were utilized with additional positive controls performed to confirm specificity and robust amplification for each assay (Plasmid standards, plus K562 or patient samples with known mutations of interest). For RQ-PCR runs using patient samples, no template control wells were negative. In addition, the PCR efficiency, slope and R2 for each assay was assessed with each batch of patient samples and was within acceptable range (e.g. WT1 n=7 batches: efficiency range 89-105%, average 94.4, R^2 range 0.993-0.999 average 0.996, slope range -3.2 - -3.62 average -3.46). Standard deviation of plasmid standard replicates for each assay was below 0.5 CT and standard values did not vary more than 1 CT between experiments or the results were discarded and the experiment was repeated as pre-specified. In addition, limit of detection for each assay was established using reference standards of known copy number, spike in experiments with leukemia cell line (K562) cDNA or cDNA from a patient sample harboring a known mutation of interest. Reference standards of known concentration were run for each gene with every batch of patient samples. All MRD calls were made while blind to clinical relapse and cytogenetics information unless otherwise specified. Detailed information for each assay is described below.

WT1 RQ-PCR

Normalized WT1 Copy number was assessed using the European LeukemiaNet WT1 and ABL1 assays **(Table S2).** Reactions were carried out using Taqman Universal Mastermix (Life technologies Carlsbad CA) in a 25µL reaction. Copies were assigned using plasmid standards of known copy number for WT1 and ABL1 genes. MRD levels are reported as the normalized copy number of WT1 transcripts per 10^4 ABL transcripts (WT1/ABLx10^4). Limit of detection and copy number values for WT1 and ABL1 assays were assigned by plasmid standard dilution experiments using the Ipsogen ProfileQuant WT1 kit and the limit of detection was established as 2x10^4 using spike-in experiments with a cell line expressing WT1 (K562). Samples were thermocycled in a RotorgeneQ thermocycler (Qiagen, Valencia, CA). Thermocycling conditions were 2 min at 50°C, 10 min activation at 95°C, and 50 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C.

NPM1mut MRD

Presence of mutated NPM1 was assessed using the assays for mutation types A, B, and D published by the German-Austrian acute myeloid leukemia study group. Together, these assays account for nearly 90% of AML NPM1 mutations. In order to increase the throughput of screening for NPM1 mutations using a high amount of cDNA (100ng) in each reaction, we used a multiplex approach with three mutation-specific reverse primers in triplicate, again with the ABL1 control gene in duplicate. This high throughput method was verified on a patient sample harboring a known type A insertion at presentation. RQ PCR was performed in 25μL reactions with 1.25 U AmpliTaq Gold, 1X Taqman PCR buffer, 4mM MgCl, 400nM NPM1 forward primer, 300nM type A, B, and D mutation reverse primers and 300nM FAM/MGB labeled NPM1 probe. The limit of detection of the assay based on a plasmid standard curve was determined and positivity was designated as n=3 of 3 replicates with ct values below the limit of detection of the assay (ct =35.81). All hits were verified to have sigmoidal amplification patterns. Type A CATA insertions were verified with the Ipsogen MutaQuant kit for NPM1. RotorgeneQ thermocycler (Qiagen, Valencia, CA) conditions were 2 min at 50°C, 10 min activation at 95°C, and 50 cycles of denaturation at 95°C for 15 sec and annealing/extension at 62°C. No template control wells were completely negative with any signal below the baseline for all experiments or the results were discarded and the e xperiment was repeated as pre-specified.

Multigene Array MRD

72 autograft samples (18 per plate) were assessed for transcript levels of ABL1, WT1, MSLN, PRAME, PRTN3, CCNA1. Plates contain internal control reactions for genomic DNA contamination, PCR efficiency and reverse transcription reaction efficiency in a 384-well custom built PCR array plate (Qiagen, Valencia, CA). Healthy control samples previously used for calculating baseline expression, cDNA from the leukemia cell line K562, and a plasmid for each target amplicon was run on each plate to ensure there were no plate printing or reagent-derived batch effects with each of the 6 plates used in this study. Plates utilized 10μL PCR reactions for each assay with SYBR Green ROX chemistry and were thermocycled on an ABI 7900 (Applied Biosystems, Foster City, CA). PCR Conditions were 2 min at 50°C, 10 min activation at 95°C, and 50 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C. Gene expression was assessed relative to the ABL1 housekeeping gene. These expression (Δct) values are plotted against expression seen in normal controls. In this study, thresholds were set relative to the GCSF stimulated peripheral blood healthy donor cohort from UCSF; the threshold was one half standard deviation above the maximum level seen.

Chromosomal Translocations

All 72 samples were assayed for the presence of chromosomal translocations Inv(16)(p13q22) (CBFB-MYH11) Type A (Expected frequency 88%), t(8;21)(q22;q22) (AML1-ETO), t(15;17)(q22;q21), (PML-RARA) breakpoint cluster 1 and 3 (expected frequency bcr1- 55%, bcr3 - 40%) while blinded to the cytogenetic profile of each patient. Samples were screened using 45ng RNA equivalent cDNA per reaction in technical replicates for each assay **(Table S2),** with Taqman Universal Mastermix (Life Technologies, Carlsbad CA) in a 10µL reaction using ABL1 as a control gene. After being un-blinded to patient cytogenetic information (see figure 4), we further interrogated samples harboring chromosomal translocations at presentation using 100ng of cDNA in triplicate for genes of interest with ABL1 as a control gene in 25µL reactions. Positivity was defined as a positive PCR signal with Ct < 36 in 3 of 3 technical replicates. RotorgeneQ thermocycler (Qiagen, Valencia, CA) conditions were 2 min at 50°C, 10 min activation at 95°C, and 50 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C.

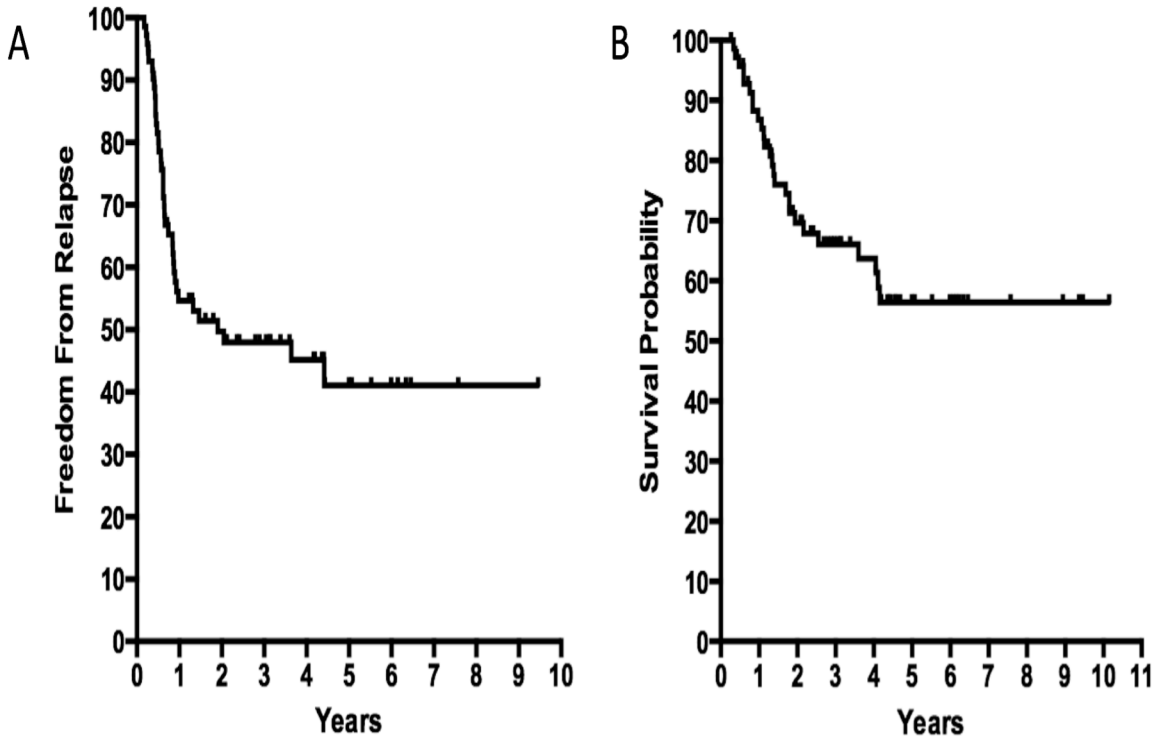
Statistical Analysis

Continuous data are reported as mean (± SD), and time data are reported as median (range). Categorical data are reported as frequency (%). Multivariate analyses of clinical data were performed using STATA SE 9 (StataCorp LP, College Station, TX)). A *p*-value of <0.05 was considered significant. Kaplan-Meier curves were generated using GraphPad Prism (GraphPad Software, La Jolla, CA) and statistical differences assessed via Log-Rank and Wilcoxon analyses. Multivariate analysis of RQ-PCR data was performed with JMP (SAS, Cary, NC). Inferential statistical analysis of RQ-PCR data was performed with Graphpad Prism. RQ-PCR data analyzed with Rotor-Gene Q series Software 2.1.0 (Qiagen, Valencia, CA) or SDS 2.3 (Applied Biosystems, Foster City, CA).

Clinical Information

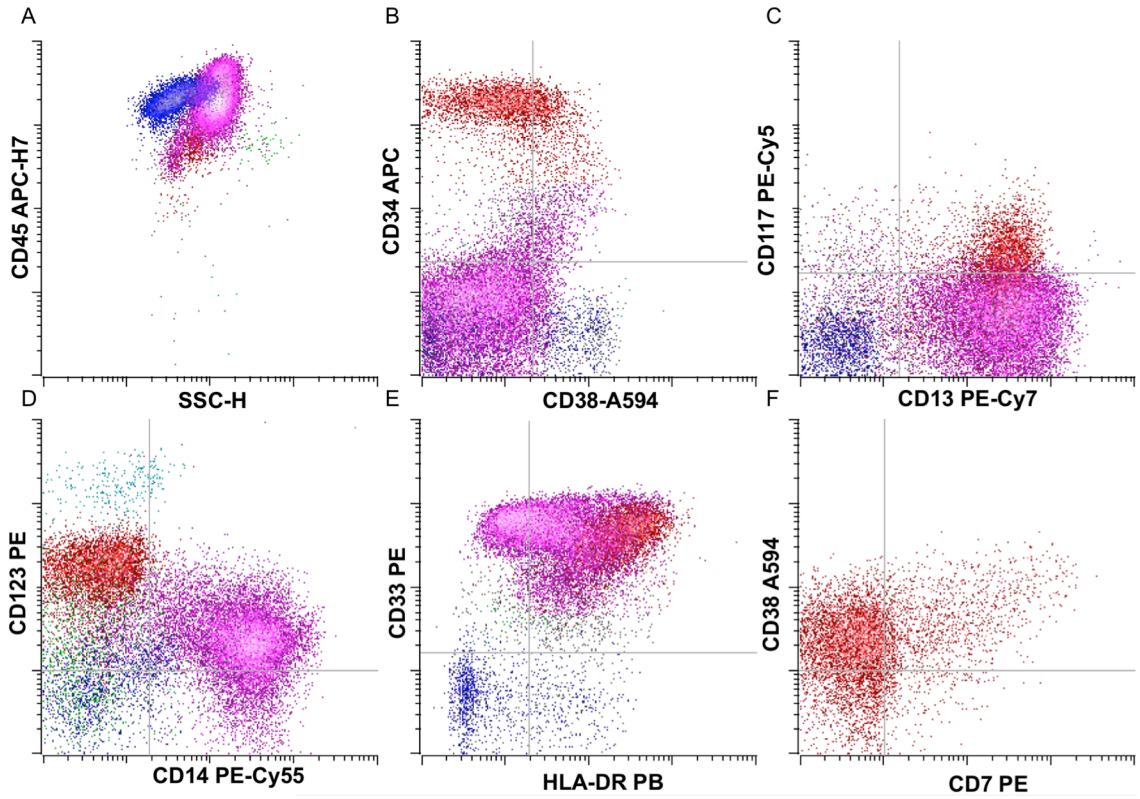
Patient histories were obtained retrospectively via two electronic medical record systems as well as via physical chart review. Two publicly available, online death indices—the Social Security Death Index (1935-2014) and the California Death Index (1940-1997)—were accessed to ensure accurate dates of death, with censoring of data on March 1, 2015. The study was approved by the UCSF Committee on Human Research with a waiver for the requirement of individual HIPAA authorization based on minimal risk. Freedom from relapse was defined as the interval between AutoHCT and relapse, as determined by standard criteria. Overall survival was defined as the interval between AutoHCT and last follow-up. Patients were determined to have therapy-related myeloid neoplasm (t-MN) rather than AML relapse if the diagnosis was made 18 months after AutoHCT and the t-MN harbored complex cytogenetics or other chromosomal abnormalities typical of secondary AML.

SUPPLEMENTAL FIGURES

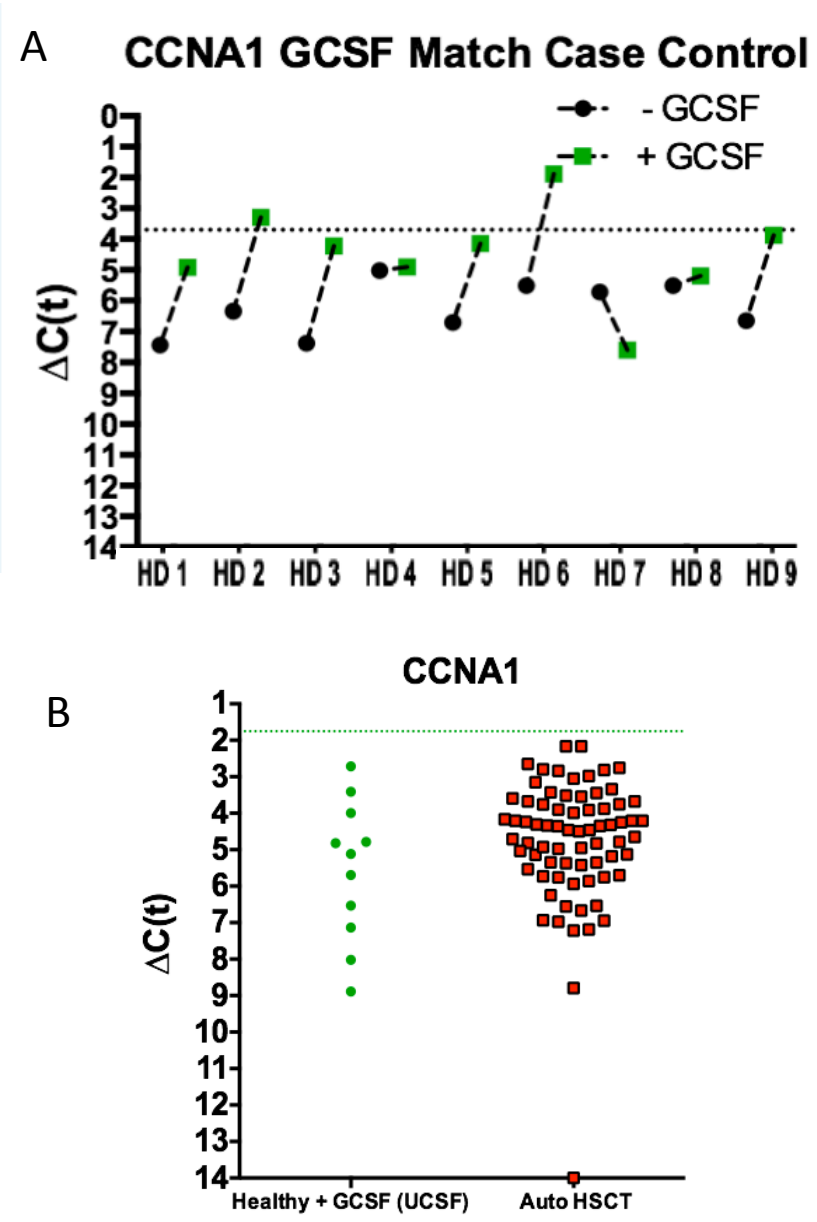


Supplemental Figure 1: Patient Survival Information

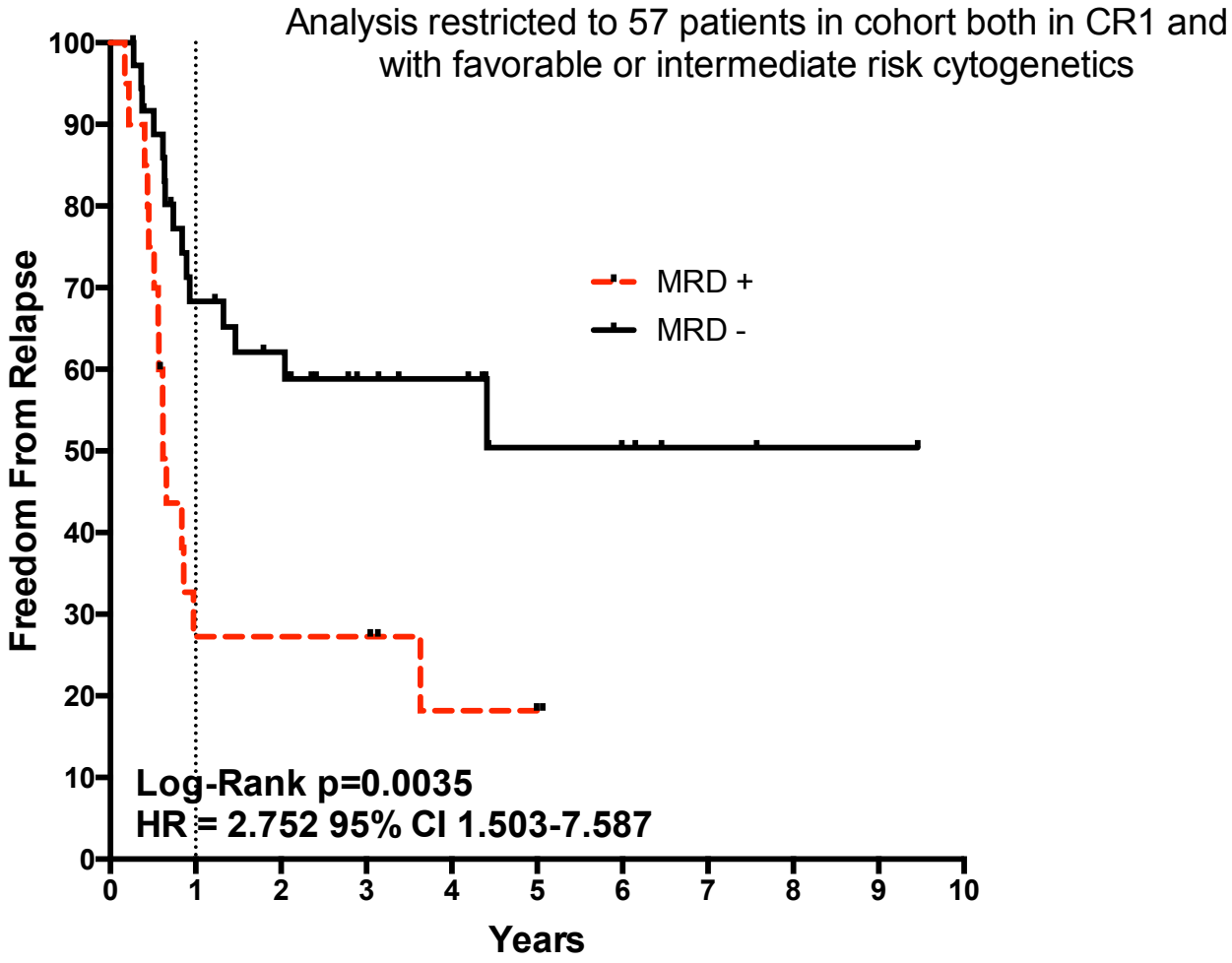
(A) Freedom from Relapse and (B) Overall Survival. The outcomes of this cohort appear representative of the recent experience at this center (UCSF) with overall FFR at 1yr (55%) and 5yr(41%) and OS at 1yr (85%) and 5yr (56%). The majority of patients (65%) had intermediate cytogenetic risk, of whom 54% remained alive and 39% remained relapse-free at five years post transplant.



Supplemental Figure 2: Leukemia immunophenotype is not detected by Flow Cytometry in the Autograft. Representative sample shown. A) Mixture of lymphocytes (blue) and expanded monocytes (magenta) with a small subset of CD34-positive progenitors (red) detected. B) The CD34-positive progenitors consist largely of a stem cell-like population (low to absent CD38) without significant maturation to lineage-committed forms. However, in contrast to the similar population seen in resting bone marrow, increased expression of D) CD123 and E) CD33 was noted on the CD34-positive progenitors (often seen in actively regenerating marrow after chemotherapy, particularly when growth factors are used). F) In at least 10% of the samples unusual CD7 expression was noted on a subset of the CD34-positive progenitors that extended to more immature forms having low CD38, this was judged to be a consequence of the process used to mobilize the stem cells (GCSF) rather than an immunophenotypic abnormality. All these findings are typical of stem cell products of this type at the center analyzing the data (Fred Hutch Cancer Center). In no sample was a discrete population having an immunophenotype different from the above CD34-positive progenitors and mature monocytes identified.



Supplemental Figure 3: CCNA1 is suboptimal for AML MRD detection in Autograft samples. While previously shown to have utility for AML MRD detection in peripheral blood samples taken prior to allogeneic transplantation (Goswami *et. al.*, 2015, ref 19) it had no use in this autograft setting. A) CCNA1 expression increases, in NIH healthy donors, following treatment with GCSF. B) CCNA1 levels in autografts from AML patients did not exceed levels seen in autografts from healthy donors.



	All Patients	CR1, Int and Fav Risk Only
N=	72	57
MRD+ (1yr FFR)	28%	27%
MRD- (1yr FFR)	67%	68%
Log-rank (Mantel-Cox)	0.0023	0.0035
Hazard Ratio	2.614	2.752
HR 95% CI	1.530-6.839	1.503-7.7587

Supplemental Figure 4: Restricting analysis to subset of patients in CR1 with favorable and intermediate risk cytogenetics does not change impact of MRD. Eight patients in the original cohort of 72 analyzed in this work were not in CR1 at the time of transplant of whom two also had poor risk or unknown cytogenetics. In addition seven patients were in CR1 but had poor risk cytogenetics (n=2), secondary AML (n=2) or had unknown cytogentics (n=3). (See Table 1 and Supplemental Table 1) Excluding these 15 patients from the analysis did not change the impact of autograft MRD detection on post-transplant outcomes.

Supplemental Table 1 Expanded patient characteristics

Patient ID	Sex	Cytogenetics/ Molecular	Primary Cyto Code 0=Unk 1=Int 2=CBF 3=Poor 4=Secondary 5=M3	Remission type	Protocol	CD34+ Dose x10^6/kg	Age at Transplant	OS	Relapsed	RFS
1	F	46,XX,t(8;21)(q22;q22)[20]	2	PIF	9302	unk	27	0.38	YES	**0.24**
2	F	normal	1	CR1	9302	2.63	54	0.33	YES	**0.28**
3	F	normal	1	CR1	9302	6.34	28	2.16	YES	**0.44**
4	F	normal	1	CR1	9302	12.50	49	1.40	YES	**0.51**
5	F	unk	0	CR2	9302	5.61	52	1.34	YES	**0.43**
6	F	11q23	3	CR1	9302	unk	64	1.06	YES	**0.44**
7	F	normal	1	CR1	9302	3.94	64	7.57	NO	**7.57**
8	F	normal	1	CR1	CALGB 19808	8.77	42	5.07	NO	**5.07**
9	M	unk	0	CR1	9302	7.02	44	5.52	NO	**5.52**
10	F	normal	1	CR1	TargBu	10.05	57	1.94	YES	**0.84**
11	M	normal	1	CR1	CALGB 10503	22.12	41	0.83	YES	**0.66**
12	F	t(8;21), ckit (+)	2	CR1	9302	7.30	28	1.80	NO	**1.80**
13	M	normal	1	CR1	TargBu	8.28	36	3.05	NO	**3.05**
14	F	normal	1	CR1	9302	35.40	53	1.28	YES	**0.41**
15	F	normal	1	CR1	TargBu	33.36	58	4.36	NO	**4.36**
16	M	normal	1	CR1	9302	4.98	61	4.40	NO	**4.40**
17	M	47,XY,+10,t(11;12)(q13;p13)[6]/46,XY[12]	1	CR1	2901	7.93	64	4.55	YES	**3.64**
18	M	normal	1	CR1	TargBu	11.49	54	1.33	YES	**1.33**
19	M	normal	1	CR1	9302	5.64	38	4.58	YES	**0.74**
20	M	46, XY, with rearrangement of 1 and 11 [20 of 20 c	3	CR1	9302	14.78	28	1.30	NO	**1.30**
21	M	unk	0	CR1	unk	8.44	45	1.79	YES	**0.87**
22	F	46 XX, t(8;21)(q22;q22)[19]/46 XX[1]	4	CR1	unk	2.34	66	3.60	NO	**3.60**
23	M	normal	1	CR1	9302	11.02	54	0.58	NO	**0.58**
24	F	normal	1	CR1	9302	9.25	69	4.11	YES	**2.05**
25	F	t(8;21) by FISH	2	CR1	9302	33.80	48	2.12	NO	**2.12**
26	F	t(1;11)	1	CR1	9302	4.22	34	0.39	NO	**0.39**
27	M	normal	1	CR1	9302	7.56	64	0.98	YES	**0.57**
28	F	t(8;21)	2	CR1	9302	3.32	60	2.69	YES	**0.98**
29	F	normal	1	CR1	9302	8.03	44	0.59	YES	**0.22**
30	M	inv(16), trisomy 8, trisomy 22	2	CR1	9302	9.08	41	0.60	YES	**0.52**
31	M	trisomy 21	1	CR1	TargBu	5.50	61	2.96	YES	**0.90**
32	F	t(8;21)	2	CR1	TargBu	6.88	29	3.13	NO	**3.13**
33	F	t(13;21) in 75% of metaphases	1	CR1	TargBu	9.21	53	3.14	NO	**3.14**
34	F	normal	2	CR3	9302		26	0.47	YES	**0.47**
37	F	normal	1	CR1	9302	13.29	57	0.83	YES	**0.36**
38	F	t(9;11)	1	CR1	TargBu	10.06	39	1.12	YES	**0.38**
39	M	derivative (13;14) robertsonian translocation	1	CR1	TargBu	7.96	24	4.15	YES	**0.86**
40	M	trisomy 8	1	CR1	9302	9.83	41	4.43	NO	**4.43**
41	F	normal	1	CR1	9302	11.04	52	5.99	NO	**5.99**
42	M	t(8;21)	2	CR1	9302	10.90	24	6.07	YES	**0.56**
43	M	normal	1	CR1	9302	6.34	48	6.15	NO	**6.15**
44	F	normal	1	CR1	9302	7.45	40	1.38	YES	**0.62**
45	M	normal	1	CR1	9302	38.13	67	5.00	NO	**5.00**
46	M	inv16	2	CR1	9302	unk	44	6.23	YES	**0.62**
47	M	normal	1	CR2	9302	10.59	25	2.55	YES	**0.26**
48	F	unk	0	CR1	9302	10.77	37	6.34	NO	**6.34**
49	M	inv16, tri22,	2	CR1	9302	6.42	30	3.38	NO	**3.38**
50	M	normal	1	CR1	9302	10.14	37	6.46	NO	**6.46**
51	M	inv16,+8	2	CR1	9302	17.26	35	0.71	NO	**0.71**
52	M	t(8;21)	2	CR1	9302	unk	33	2.36	NO	**2.36**
53	F	normal	1	CR1	9302	3.80	69	1.13	YES	**0.64**
54	F	normal	1	CR1	9302	12.27	54	4.69	YES	**0.17**
55	M	normal	1	CR1	9302	10.80	55	1.23	NO	**1.23**
56	F	normal	1	CR1	9302	10.31	34	1.79	YES	**0.93**
57	F	normal	1	CR1	9302	8.38	43	2.41	NO	**2.41**
58	M	inv(16)(p13.1q22)[20]0	2	CR1	9302	unk	30	2.89	NO	**2.89**
59	F	t(11;12)	1	CR1	CALGB 19808		50	4.19	NO	**4.19**
60	M	normal	1	CR1	CALGB 19808	13.12	43	4.05	YES	**1.47**
61	M	trisomy 6	1	CR1	9302	61.88	26	9.36	YES	**0.84**
62	M	trisomy 8, -Y	1	CR1	9302	9.02	58	1.69	YES	**0.45**
63	F	normal	1	CR1	9302	11.80	57	9.46	NO	**9.46**
65	F	normal	1	CR2	9302	8.27	36	1.88	NO	**1.61**
66	M	46,XX,t(X;19)(q23;p13.1)[20]	1	CR2	9302	unk	60	4.19	NO	**4.19**
67	F	normal	1	CR1	9302	13.81	51	8.94	YES	**4.41**
68	F	13a, 14q, 20q del	3	CR2	9302	9.77	56	0.41	NO	**0.41**
69	F	normal	4	CR1	CALGB 19808	9.60	57	4.16	NO	**4.16**
71	F	inv(16)	2	CR1	9302	11.51	48	10.15	YES	**0.62**
72	M	positive PML/RARA, 46 XY	5	CR2	9302	5.90	62	4.05	YES	**1.90**
73	M	inv16	2	CR1	9302	7.14	47	0.27	NO	**0.27**
74	M	normal	1	CR1	9302	6.50	45	2.08	NO	**2.08**
75	F	47, XX, +8, i(8)(q10)x2[17]/47, XX, +6[2]/46, XX[1	1	CR1	9302	3.00	65	0.76	YES	**0.64**
76	F	normal	1	CR1	9302	10.57	57	2.79	NO	**2.79**

Supplemental Table 2 High throughput RQ-PCR array SYBR green assays for WT1, PRAME, PR3, CCNA1, MSLN, and ABL1. (Qiagen).

A)

Symbol	Description	Unigene No	Refseq No	Band Size (bp)	Reference Position	NCBI Link
WT1	Wilms tumor 1	Hs.591980	NM_000378	162	674	http://www.ncbi.nlm.nih.gov/gene?term=NM_000378&doptcmdl=Graphics&cmd=search
PRAME	Preferentially expressed antigen in melanoma	Hs.30743	NM_006115	135	1290	http://www.ncbi.nlm.nih.gov/gene?term=NM_006115&doptcmdl=Graphics&cmd=search
PRTN3	Proteinase 3	Hs.928	NM_002777	82	653	http://www.ncbi.nlm.nih.gov/gene?term=NM_002777&doptcmdl=Graphics&cmd=search
CCNA1	Cyclin A1	Hs.417050	NM_003914	120	1571	http://www.ncbi.nlm.nih.gov/gene?term=NM_003914&doptcmdl=Graphics&cmd=search
MSLN	Mesothelin	Hs.408488	NM_005823	119	1267	http://www.ncbi.nlm.nih.gov/gene?term=NM_005823&doptcmdl=Graphics&cmd=search
ABL1	C-abl oncogene 1, non-receptor tyrosine kinase	Hs.431048	NM_005157	81	5253	http://www.ncbi.nlm.nih.gov/gene/25

B) Primer and Probe sequences used for Real-Time Quantitative PCR used in this study.

Assay	Assay Reference	Forward Primer Sequence 5'-3'	Reverse Primer Seqence 5'-3'	Probe sequence 5'-3'
NPM1 Type A	Krönke et al	GAA GAA TTG CTT CCG GAT GAC T	CTT CCT CCA CTG CCA GAC AGA	FAM-ACCAAGAGGCTATTCAAGAT-MGB
NPM1 Type B	Krönke et al	GAA GAA TTG CTT CCG GAT GAC T	TTC CTC CAC TGC CAT GCA G	FAM-ACCAAGAGGCTATTCAAGAT-MGB
NPM1 Type D	Krönke et al	GAA GAA TTG CTT CCG GAT GAC T	TTC CTC CAC TGC CCT GCA G	FAM-ACCAAGAGGCTATTCAAGAT-MGB
Inv(16)(p13q22) (CBFB-MYH11)	Gabert et al	CAT TAG CAC AAC AGG CCT TTG A	AGG GCC CGC TTG GAC TT	6-FAM-TCG CGT GTC /ZEN/CTT CTC CGA GCC T-IABkFQ
t(8;21)(q22;q22) (AML1-ETO)	Gabert et al	CAC CTA CCA CAG AGC CAT CAA	ATC CAC AGG TGA GTC TGG CAT T	6-FAM-AAC CTC GAA /ZEN/ATC GTA CTG AGA AGC ACT CCA-IABkFQ
t(15;17)(q22;q21) (PML-RARA) bcr1	Gabert et al	TCT TCC TGC CCA ACA GCA A	GCT TGT AGA TGC GGG GTA GAG	6-FAM-AGT GCC CAG /ZEN/CCC TCC CTC GC-IABkFQ
t(15;17)(q22;q21) (PML-RARA) bcr3	Gabert et al	CCG ATG GCT TCG ACG AGT T	GCT TGT AGA TGC GGG GTA GAG	6-FAM-AGT GCC CAG /ZEN/CCC TCC CTC GC-IABkFQ
WT1	Cilloni et al	CGC TAT TCG CAA TCA GGG TTA	GGG CGT GTG ACC GTA GCT	Cy5-AGC ACG GTC ACC TTC GAC GGG A-IAbRQSp
ABL1	Beillard et al	TGG AGA TAA CAC TCT AAG CAT AAC TAA AGG T	GAT GTA GTT GCT TGG GAC CCA	5HEX-CCA TTT TTG /ZEN/GTT TGG GCT TCA CAC CAT T-IABkFQ

Supplemental Table 3 Essential Minimum Information for Publication (MIQE) requirements met in this study.

ESSENTIAL ITEMS of Minimum Information for Publication (MIQE) for RQ-PCR Experiments	IMPORTANCE	CHECKLIST
EXPERIMENTAL DESIGN		
Definition of experimental and control groups	ESSENTIAL	**YES**
Number within each group	ESSENTIAL	**YES**
SAMPLE		
Description	ESSENTIAL	**YES**
Microdissection or macrodissection	ESSENTIAL	NA
Processing procedure	ESSENTIAL	**YES**
If frozen - how and how quickly?	ESSENTIAL	**YES**
If fixed - with what, how quickly?	ESSENTIAL	NA
Sample storage conditions and duration (especially for FFPE samples)	ESSENTIAL	**YES**
NUCLEIC ACID EXTRACTION		
Procedure and/or instrumentation	ESSENTIAL	**YES**
Name of kit and details of any modifications	ESSENTIAL	**YES**
Details of DNase or RNAse treatment	ESSENTIAL	**YES**
Contamination assessment (DNA or RNA)	ESSENTIAL	**YES**
Nucleic acid quantification	ESSENTIAL	**YES**
Instrument and method	ESSENTIAL	**YES**
RNA integrity method/instrument	ESSENTIAL	**YES**
RIN/RQI or Cq of 3' and 5' transcripts	ESSENTIAL	**YES**
Inhibition testing (Cq dilutions, spike or other)	ESSENTIAL	**YES**
REVERSE TRANSCRIPTION		
Complete reaction conditions	ESSENTIAL	**YES**
Amount of RNA and reaction volume	ESSENTIAL	**YES**
Priming oligonucleotide (if using GSP) and concentration	ESSENTIAL	**YES**
Reverse transcriptase and concentration	ESSENTIAL	**YES**
Temperature and time	ESSENTIAL	**YES**
qPCR TARGET INFORMATION		
If multiplex, efficiency and LOD of each assay.	ESSENTIAL	NA
Sequence accession number	ESSENTIAL	**YES**
Amplicon length	ESSENTIAL	**YES**
In silico specificity screen (BLAST, etc)	ESSENTIAL	**YES**
Location of each primer by exon or intron (if applicable)	ESSENTIAL	**YES**
What splice variants are targeted?	ESSENTIAL	NA
qPCR OLIGONUCLEOTIDES		
Primer sequences	ESSENTIAL	**YES**
Location and identity of any modifications	ESSENTIAL	**YES**
qPCR PROTOCOL		
Complete reaction conditions	ESSENTIAL	**YES**
Reaction volume and amount of cDNA/DNA	ESSENTIAL	**YES**
Primer, (probe), Mg++ and dNTP concentrations	ESSENTIAL	**YES**
Polymerase identity and concentration	ESSENTIAL	**YES**
Buffer/kit identity and manufacturer	ESSENTIAL	**YES**
Additives (SYBR Green I, DMSO, etc.)	ESSENTIAL	**YES**
Complete thermocycling parameters	ESSENTIAL	**YES**
Manufacturer of qPCR instrument	ESSENTIAL	**YES**
qPCR VALIDATION		
Specificity (gel, sequence, melt, or digest)	ESSENTIAL	**YES**
For SYBR Green I, Cq of the NTC	ESSENTIAL	**YES**
Standard curves with slope and y-intercept	ESSENTIAL	**YES**
PCR efficiency calculated from slope	ESSENTIAL	**YES**
r2 of standard curve	ESSENTIAL	**YES**
Linear dynamic range	ESSENTIAL	**YES**
Cq variation at lower limit	ESSENTIAL	**YES**
Evidence for limit of detection	ESSENTIAL	**YES**
If multiplex, efficiency and LOD of each assay.	ESSENTIAL	NA
DATA ANALYSIS		
qPCR analysis program (source, version)	ESSENTIAL	**YES**
Cq method determination	ESSENTIAL	**YES**
Outlier identification and disposition	ESSENTIAL	**YES**
Results of NTCs	ESSENTIAL	**YES**
Justification of number and choice of reference genes	ESSENTIAL	**YES**
Description of normalisation method	ESSENTIAL	**YES**
Number and stage (RT or qPCR) of technical replicates	ESSENTIAL	**YES**
Repeatability (intra-assay variation)	ESSENTIAL	**YES**
Statistical methods for result significance	ESSENTIAL	**YES**
Software (source, version)	ESSENTIAL	**YES**