Supplemental Table 1: NHLBI Transplant Protocols

Protocol	n	Stem Cell Source	Conditioning Intensity	Total Fludarabine mg/m2	Total Cytoxan m/kg	Other conditioning	TBI cGy	TBI (lung)	Unselected T cell addback	Pre-DLI GVHD Prophylaxis
93-H-0212	1	Marrow	Fully ablative	0	120	none	1360	1360	d30:2 x10e^6/kg; d45: 5x10e^7/kg	Standard dose CSA (200-400) day -4 to day +180
94-H-0092	3	Marrow	Fully ablative	0	120	none	1360	1360	d30:2 x10e^6/kg; d45: 5x10e^7/kg	Standard dose CSA (200-400) day -4 to day +180
97-H-0099	9	PBSC	Fully ablative	0	120	none	1360	1360	d45:1 x10e^7/kg; d100 (not for CML): 5x10e^7/kg	Standard dose CSA (200-400) day -4 to day +180
99-H-0046	11	PBSC	Fully ablative	0	120	none	1360	1360	d45:1 x10e^7/kg; d100 (not for CML): 5x10e^7/kg	Low dose CSA (100-200) day -4 to day +120 in the first 20 patients , no CSA in last 20 patients
01-H-0162	3	PBSC	Non-myeloablative	125	/	variable	0	0		Standard dose CSA (200-400) day -4 to >day +30
02-H-0111	6	PBSC	Fully ablative	125	120	none	1200	900	d45:1 x10e^7/kg; d100: 2x10e^7/kg	No CSA initially, Cyclosporine 6 mg/kg/day by mouth, dose adjusted to achieve levels 100-200 ng/ml day 44 to day +120
03-H-0192	1	PBSC	Fully ablative	125	120	25 Gy irradiated donor lymphocytes 2 x 10e8/kg day -4	1200	600	day 45, 1x 10e7/kg	No CSA initially, Cyclosporine 6 mg/kg/day by mouth, dose adjusted to achieve levels 100-200 ng/ml day 44 to day +120
04-H-0112	17	PBSC	Fully ablative	125	120	VP16 (60 mg/kg) for high risk-AML w blast >10%, ALL in CR2 or higher.	1200	600	day 60, 1x 10e7/kg	Low dose CSA (100-200) day -6 to day +21
06-H-0248	17	PBSC	Fully ablative for age <55 (n=13), Reduced Intensity for Age >55 (n=4)	125	120	none	1200 for Age <55, 600>55	600	d90, 5 x 10e6/kg	Low dose CSA (100-200) day -6 to day +21
12-H-0028	4	PBSC	Fully ablative for age <55 (n=4), Reduced Intensity for Age >55 (n=0)	125	120	none	1200 for Age <55, 600>55	600	none scheduled	Low dose CSA (100-200) day -6 to day +21

N.B: Second column ("n") lists number of patients for whom pre-SCT samples could be identified. In addition to above, two protocols enrolled a single patient – 94-H-0010 (syngeneic from twin) and 98-H-0122 (haplo-identical). Clinical outcomes from these transplants were consistent with the pattern described in the text.

Supplemental Table 2: Additional Patient Characteristics

Non-relapse mortality (n=22)

- Median age/sex: 39 years, 64% Female
- In patients with CR at allo-SCT: 15/48 (31%)
- If active disease at allo-SCT: 7/26 (27%)

Relapse within 1yr after allo-SCT (n=24)

- Median age/sex: 41 Years, 50% Female
- In patients with CR at allo-SCT: 9/48 (19%)
- If active disease at allo-SCT: 15/26 (58%)

Relapse within 2yr after allo-SCT (n=26)

- Median age/sex: 40 years, 50% Female
- In patients with CR at allo-SCT: 10/48 (21%)
- If active disease at allo-SCT: 16/26 (62%)

Relapse after allo-SCT (n=28)

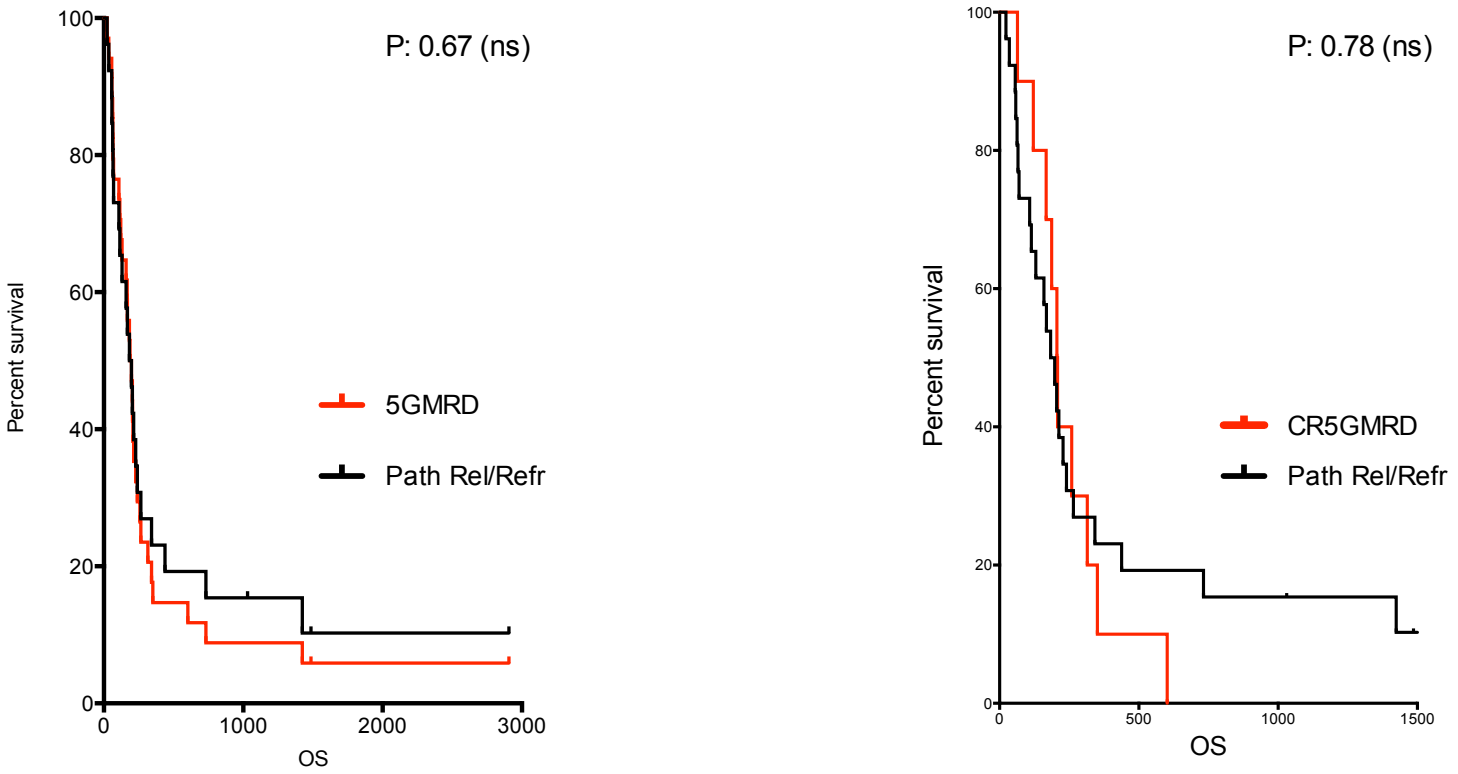
- Median age/sex: 40 years, 54% Female
- In patients with CR at allo-SCT: 11/48 (23%)
- If active disease at allo-SCT: 17/26 (65%)

Patients alive after allo-SCT (n=25)

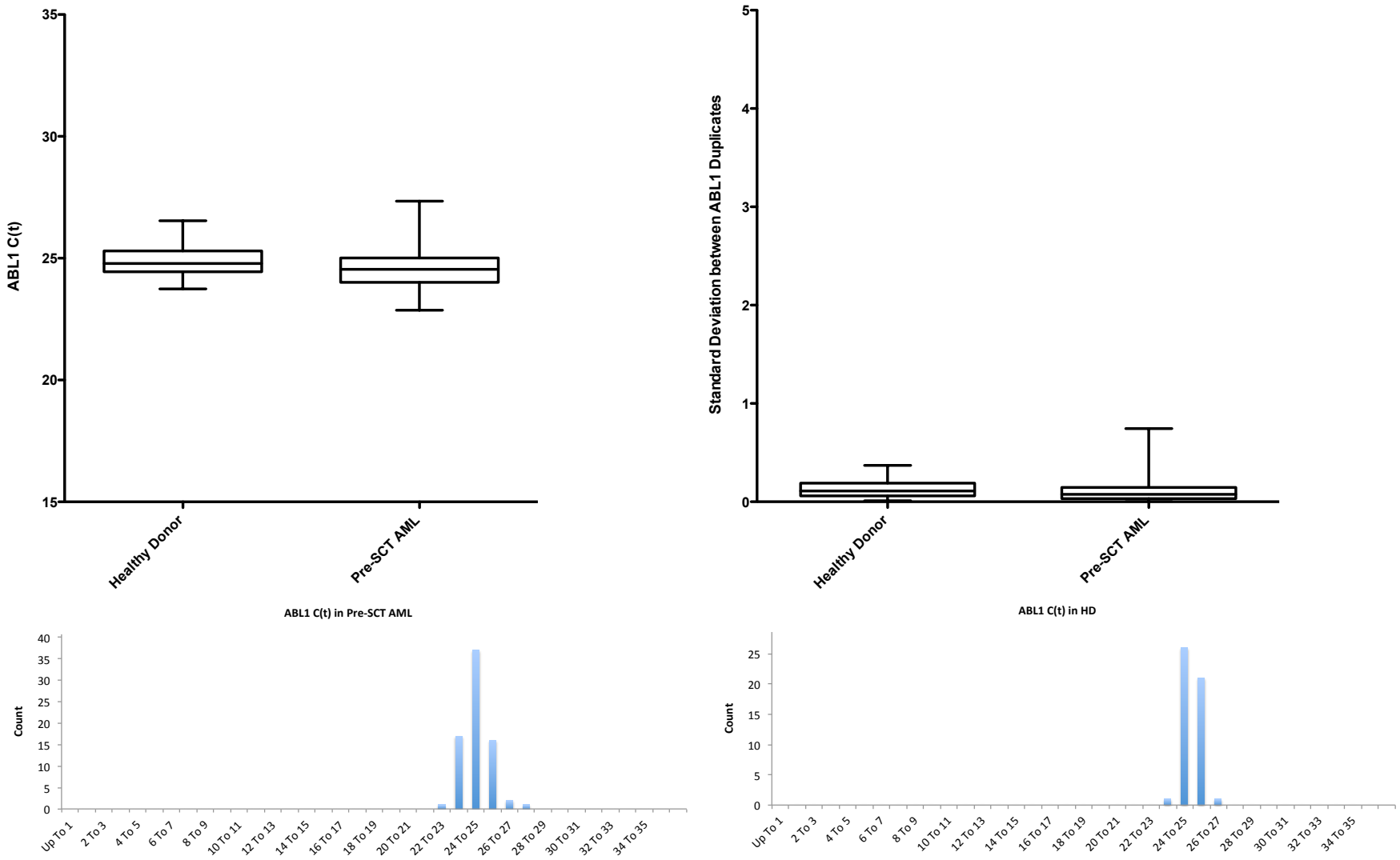
- Median age/sex: 44 years, 48% Female
- In patients with CR at allo-SCT: 22/48 (46%)
- If active disease at allo-SCT: 3/26 (12%)

Supplemental Table 3: Gene targets used in multigene MRD assay.

Symbol	Description	Unigene No	Refseq No	Band Size (bp)	Reference Position
WT1	Wilms tumor 1	Hs.591980	NM_000378	162	674
PRAME	Preferentially expressed antigen in melanoma	Hs.30743	NM_006115	135	1290
PRTN3	Proteinase 3	Hs.928	NM_002777	82	653
CCNA1	Cyclin A1	Hs.417050	NM_003914	120	1571
MSLN	Mesothelin	Hs.408488	NM_005823	119	1276
ABL1	C-abl oncogene 1, non-receptor tyrosine kinase	Hs.431048	NM_005157	81	5253

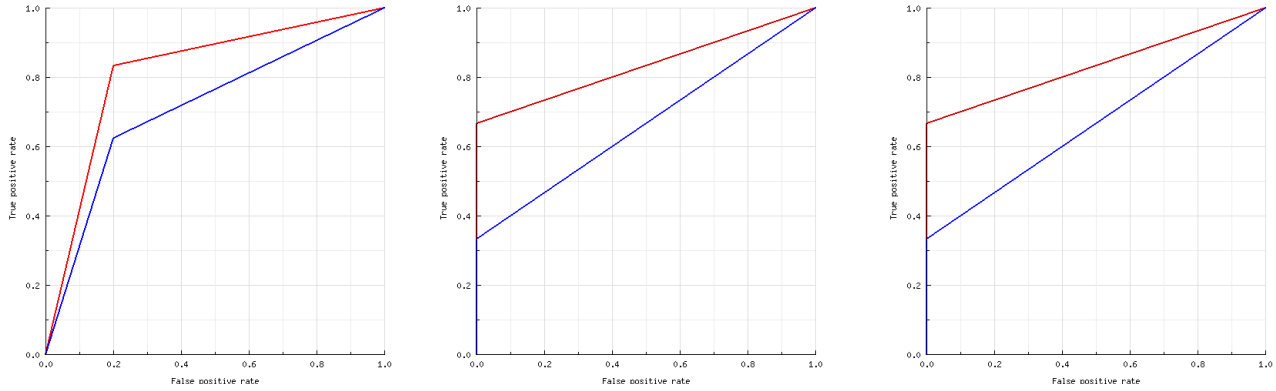


Supplemental Figure 1: Post transplantation survival statistically indistinguishable between 5GMRD patients and those with pathologist clinical diagnosis of relapsed/ refractory active disease (Path Rel/Refr). Both all patients with 5G-MRD positive test from peripheral blood (5GMRD, left graph) and the subset who were 5G-MRD positive and also were judged to be in a pathological complete remission (CR5GMRD, right graph) had survival not different from those with active disease. Curve comparison by log-rank Mantel-Cox test.



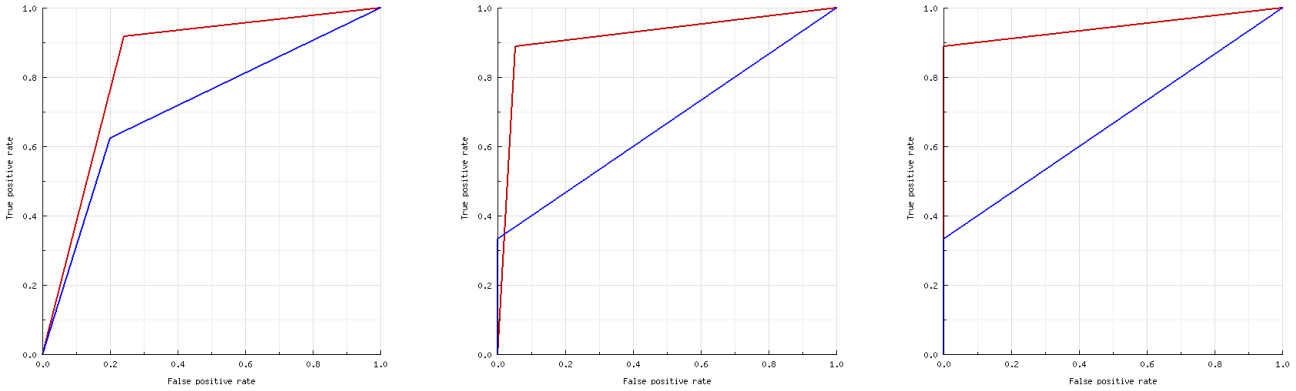
Supplemental Figure 2: Multigene AML MRD quantitative RT-PCR array is highly reproducible. Upper Left: One ug of total RNA was used for each sample timepoint, box plots whiskers represent minimium and maximum *ABL1* C(t) observed. Upper Right: Standard deviation of technical replicates of *ABL1* (performed in duplicate on each sample). Lower: Distribution of ABL1 C(t) values in pre-SCT AML patients (bottom left) and healthy donors (bottom right)

A) **Receiver operating characteristic (ROC) Curves: WT1 (Blue) vs 4G MRD (Red)**



	74 pts (all)	48 pts (all CR)	37 pts (CR exclude NRM)
WT1 AUC	0.7125	0.6667	0.6667
4G AUC	0.8167	0.8333	0.8333
AUC diff	-0.142	-0.1667	-0.1667
Conf. Interval	-0.18715, -0.02118	-0.33000, -0.00334	-0.33000, -0.00334
p-value	**0.0139**	**0.0455**	**0.0455**

Receiver operating characteristic (ROC) Curves: WT1 (Blue) vs 5G MRD (Red)



	74 pts (all)	48 pts (all CR)	37 pts (CR exclude NRM)
WT1 AUC	0.7125	0.6667	0.6667
5G AUC	0.8383	0.9188	0.9444
AUC diff	-0.1258	-0.2521	-0.2778
Conf. Interval	-0.22268, -0.02899	-0.42784 , -0.07644	-0.44994, -0.10561
p-value	**0.0109**	**0.0049**	**0.0016**

B) **McNemar's Test** 37 patients (CR excluding NRM)

Sensitivity (relapse, n=9)

	5G MRD+	**5G MRD-**
WT1+	3	0
WT1-	5	1

Difference: -0.556, **p: 0.031** (1 sided)

Specificity (no-relapse, n=28)

	5G MRD+	**5G MRD-**
WT1+	0	0
WT1-	0	28

Difference: 0, p: 0.5 (1 sided)

Supplemental Figure 3: Sensitivity of Multigene MRD test is statistically superior to WT1 MRD alone. *See over for complete legend.*

Supplemental Figure 3: Sensitivity of Multigene MRD test is statistically superior to WT1 MRD alone. A) Performance characteristics of both WT1 and MG-MRD displayed by receiver operating characteristic curves, demonstrating the statistically significant difference between the tests. B) WT1 is a constituent part of the MG-MRD assay, and by design a positive level of expression for any gene is considered a positive MG-MRD test (*ie: not possible for addition of extra gene assays to WT1 to decrease sensitivity*). A one-sided McNemar's test was therefore performed which determined the improvement in sensitivity to predict relapse in the MG-MRD test compared to WT1 alone was statistically significant (p: 0.031). Specificity was not different between the two tests.