

Early prediction of treatment outcome in acute myeloid leukemia by measurement of *WT1* transcript levels in peripheral blood samples collected after chemotherapy

Daniela Cilloni,¹ Francesca Messa,¹ Francesca Arruga,¹ Ilaria Defilippi,¹ Enrico Gottardi,¹ Milena Fava,¹ Sonia Carturan,¹ Renata Catalano,¹ Enrico Bracco,¹ Emanuela Messa,¹ Paolo Nicoli,¹ Daniela Diverio,² Miguel A. Sanz,³ Giovanni Martinelli,⁴ Francesco Lo-Coco,⁵ and Giuseppe Saglio¹

¹Dept. of Clinical and Biological Sciences, University of Turin, Italy; ²Dept. of Cellular Biotechnologies and Hematology, University La Sapienza, Rome, Italy; ³Dept of Medical Biopathology, Hospital Universitario La Fe, Valencia, Spain; ⁴Dept. of Hematology, Seragnoli Institute, University of Bologna, Bologna, Italy and ⁵Department of Biopathology, University Tor Vergata, Rome, Italy

Citation: Cilloni D, Messa F, Arruga F, Defilippi I, Gottardi E, Fava M, Carturan S, Catalano R, Bracco E, Messa E, Nicoli P, Diverio D, Sanz MA, Martinelli G, Lo-Coco F, and Saglio G. Early prediction of treatment outcome in acute myeloid leukemia by measurement of *WT1* transcript levels in peripheral blood samples collected after chemotherapy. *Haematologica* 2008 June; 93(6):921-924.
doi: 10.3324/haematol.12165

Appendix 1. Table 1 Clinical and biological characteristics of the patients. M= male. F= female. NK =normal karyotype. ITD= Internal Tandem Duplication. WT= Wild Type. CHT regimen 1: 1= GIMEMA protocol for patients < 60 years, 2= GIMEMA protocol for patients > 60 years, 3 other chemotherapy protocols.

Pt.	sex	age	FAB	cytogenetic	FLT3	WBC	CHT regimen	WT1 at diagnosis	WT1 after Induction CHT	months of follow-up	months from diagnosis to relapse	outcome
Patients relapsed with WT1 above the normal range after induction CHT												
1	M	68	M2	NK	ITD	44200	2	13132	49	8	8	Alive in relapse
2	M	67	M4	NK	ITD	39500	2	3904	24	14	14	Deceased
3	M	65	M5	NK	WT	29000	2	786	29	7	7	Deceased
4	M	52	M5	t(6;9)	ITD	95000	3	4412	98	8	4	Deceased
5	F	64	M0	NK	ITD	1200	2	1243	168	14	12	Deceased
6	M	65	M1	complex	WT	3800	2	768	123	16	16	Deceased
7	F	42	M1	NK	WT	48000	1	486	23	7	7	Deceased
8	M	21	M2	neg	WT	3100	1	5104	220	6	6	Deceased
9	F	50	M0	inv(3)	WT	13200	1	3099	119	5	5	Deceased
10	M	52	M0	t(8;21)	WT	26400	1	4264	30	15	15	Deceased
11	M	36	M4	NK	WT	6050	1	2388	394	7	7	Deceased
12	M	40	M1	NK	ITD	29900	1	13254	43	11	11	Deceased
13	M	26	M1	NK	ITD	6000	1	23589	176	5	5	Deceased
14	F	37	M1	NK	WT	28000	1	2645	124	6	6	Deceased
15	F	41	M0	NK	WT	14700	1	21933	43	7	7	Deceased
16	M	48	M0	NK	WT	2500	1	1352	49	8	8	Deceased
17	F	45	M1	NK	WT	20600	1	2799	91	10	10	Deceased
18	M	45	M2	NK	NA	43000	1	3245	29	10	10	Deceased
19	F	20	M1	NK	ITD	120000	1	10042	1558	10	5	Deceased
20	F	67	M0	NK	WT	16200	2	10472	234	6	6	Deceased
21	F	50	M0	NK	ITD	24000	3	5764	2840	12	10	Alive in relapse
22	M	62	M1	NK	WT	29000	2	6790	112	8	6	Alive in relapse
23	M	64	M5	NK	ITD	23250	2	8240	140	8	6	Deceased
Median value						24000	4264	112	8	7		
Patients relapsed with WT1 within the normal range after induction CHT												
24	M	18	M2	t(8;21)	WT	18300	1	2364	12	10	10	Deceased
25	M	45	M2	t(8;21)	WT	14200	1	2786	1	12	12	Deceased
26	M	36	M7	complex	WT	110500	1	7425	18	9	9	Deceased
27	F	36	M2	NK	ITD	60600	1	2490	2	17	9	Deceased
28	F	19	M0	NK	ITD	65000	1	5662	15	38	16	Alive in CR 2
29	F	32	M0	NK	ITD	3280	1	673	14	18	18	Deceased
30	F	20	M2	inv16	WT	17800	1	12226	4,6	19	6	Alive in CR2
31	M	23	M4	inv16	WT	16800	1	14655	6	27	11	Alive in CR2
32	F	18	M4	inv16	WT	19500	1	54345	2	13	13	Deceased
33	F	53	M2	NK	ITD	122000	1	24614	5	15	14	Deceased
34	F	28	M1	NA	NA	500	1	1320	7	9	7	Deceased
35	M	65	M2	NK	ITD	1100	2	55	13	66	44	Alive in CR 2
36	M	38	M0	NK	ITD	1500	2	8488	8	18	17	Deceased
37	F	67	M5b	NK	WT	26900	2	2046	3	50	7	Alive in 2 CR
38	F	66	M2	NK	ITD	24000	2	1545	2	9	9	Deceased
39	F	34	M1	NK	WT	900	1	1387	6	7	6	Deceased

40	M	45	M1	NK	WT	17800	1	6455	5	11	11	Alive in 2 CR
41	M	48	M0	NK	NA	20300	1	3546	12	35	35	Deceased
42	F	48	M1	NK	WT	40200	1	12700	6	18	18	Alive in relapse
43	F	39	M5	NK	WT	32100	1	2020	14	22	18	Deceased
44	M	42	M2	NK	ITD	37800	1	9812	12	36	22	Alive in CR2
Median value						19500	3546	6	18	12		

Patients in CR with WT1 within the normal range after induction CHT

45	M	25	M2	t(8;21)	WT	70250	1	188	6	55		Alive in CR1
46	F	19	M2	t(8;21)	WT	16000	1	4181	0	26		Alive in CR1
47	M	35	M2	t(8;21)	WT	21000	1	4975	14	24		Alive in CR1
48	F	27	M1	NK	D835	2300	1	14375	3,8	17		Alive in CR1
49	M	43	M2	NK	WT	24900	1	3245	4	44		Alive in CR1
50	F	42	M0	NK	WT	1300	1	4376	13	38		Alive in CR1
51	M	44	M0	NK	WT	25800	1	4758	2	60		Alive in CR1
52	F	46	M2	NK	NA	96800	1	11253	3	28		Alive in CR1
53	M	47	M0	NK	ITD	62000	1	3264	5,6	30		Alive in CR1
54	F	50	M0	NK	ITD	1700	1	299	8,6	28		Alive in CR1
55	M	54	M1	NK	WT	6300	1	4584	0	23		Alive in CR1
56	M	51	M1	NK	WT	39200	1	15012	14	38		Alive in CR1
57	F	20	M0	NK	ITD	43200	1	789	19	37		Alive in CR1
58	F	74	M2	NK	WT	1050	2	3245	2,5	24		Alive in CR1
59	M	64	M2	NK	WT	2500	2	2557	4	41		Alive in CR1
60	M	56	M0	NK	WT	1870	3	68	10	50		Alive in CR1
61	M	45	M4	NK	NA	2980	1	97	5	30		Alive in CR1
62	M	17	M4	inv16	WT	44900	1	5670	8	35		Alive in CR1
63	F	64	M0	NK	ITD	9800	2	163	7	30		Alive in CR1
64	M	60	M4	NK	WT	160300	2	1644	12	20		Alive in CR1
65	F	49	M2	t(8;21)	WT	18300	1	8970	0,5	18		Alive in CR1
66	M	71	M5	NK	WT	1000	2	1546	6	20		Alive in CR1
67	M	54	M1	NK	ITD	980	1	1182	14	18		Alive in CR1
68	F	60	M5b	NK	WT	57200	1	1255	1,7	44		Alive in CR1
69	F	61	M0	NK	WT	26000	2	7549	4	14		Alive in CR1
70	F	64	M2	NK	WT	19200	2	5830	2	16		Alive in CR1
71	M	65	M4	NK	ITD	1300	2	2090	7	12		Alive in CR1
Median value						18300	3245	5,6	28			

Patients resistant to CHT

72	M	63	M0	NK	ITD	2340	2	95549	65501			Deceased
73	M	61	M1	NK	ITD	4590	2	5647	18452			Deceased
74	F	68	M2	NK	ITD	25200	2	986	1023			Deceased
75	F	65	M0	NK	WT	46000	2	417	230			Deceased
76	F	34	M0	t(2;3)	WT	205500	1	8796	1554			Deceased
77	M	65	M4	NK	WT	7800	2	14717	243			Deceased
78	F	67	M5	complex	WT	1020	3	238	5376			Deceased
79	M	18	M0	inv 3	WT	10200	1	13386	9804			Deceased
80	M	21	M0	NK	ITD	120900	1	7800	5300			Deceased
81	F	65	M1	NK	WT	1200	2	2300	1890			Deceased
82	M	64	M2	NK	WT	45990	2	2910	3180			Deceased
Median value						10200	5647	3180				

Clinical and biological characteristics of the patients. M: male; F: female; NK: normal karyotype. ITD: internal tandem duplication. WT: wild Type; CHT regimen 1: 1=GIMEMA protocol for patients < 60 years, 2= GIMEMA protocol for patients > 60 years, 3 other chemotherapy protocols.

Appendix 2: Chemotherapy protocols

Patients under 60 years were treated following standard protocols established by the GIMEMA Cooperative Group: a 5-day pre-treatment with hydroxyurea (HU) at 2 g/sqm/d from days - 4 to 0; Induction treatment with daunorubicin (DNR) 50 mg/m²/d on days 1, 3 and 5; cytosine-arabioside (ARA-C) 100 mg/m²/d on days 1 to 10; etoposide 100 mg/sqm/d on days 1 to 5; to be repeated in case of partial remission (PR); consolidation therapy with DNR 50 mg/m²/day on days 4 to 6 and intermediate-doses ARA-C (500 mg/m²/12 hrs. on days 1 to 6) for patients achieving CR after either the first or the second induc-

tion cycle. Post-consolidation treatment consisted of allogeneic stem cell transplantation (SCT) for patients with HLA-identical siblings, and peripheral blood stem cell autograft for patients without donors. Elderly patients were treated with two cycles of daunorubicin 45 mg/sqm/d on days 1, 3 and 5 plus cytosine arabinoside 100 mg/sqm/d on days 1 to 7.

The distribution of patients who underwent autologous and allogeneic BMT is similar in the different groups of patients, those with WT1 above the normal range who relapsed and those showing WT1 within the normal range who relapsed or persisted in CR.