

Article

Myelomonocytic Skewing in Vitro Discriminates Subgroups of Patients with Myelofibrosis with A Different Phenotype, A Different Mutational Profile and Different Prognosis

Klaus Geissler ^{1,2,*}, Bettina Gisslinger ³, Eva Jäger ⁴, Roland Jäger ⁵, Ana Iris Schiefer ⁶, Edith Bogner ⁵, Elisabeth Fuchs ⁵, Fiorella Schischlik ⁵, Donat Alpar ⁵, Ingrid-Simonitsch-Klupp ⁶, Robert Kralovics ^{4,5} and Heinz Gisslinger ³

¹ Medical School, Sigmund Freud University, 1020 Vienna, Austria

² Department of Internal Medicine V with Hematology, Oncology and Palliative Care, Hospital Hietzing, 1130 Vienna, Austria

³ Division of Hematology and Hemostaseology, Department of Internal Medicine I, Medical University of Vienna, 1090 Vienna, Austria; bettina.gisslinger@meduniwien.ac.at (B.G.); heinz.gisslinger@meduniwien.ac.at (H.G.)

⁴ Department of Laboratory Medicine, Medical University of Vienna, 1090 Vienna, Austria; eva.jaeger@akhwien.at (E.J.); rkralovics@cemm.oeaw.ac.at (R.K.)

⁵ CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, 1090 Vienna, Austria; roland.jaeger@meduniwien.ac.at (R.J.); edith_bogner@hotmail.com (E.B.); elisabeth.fuchs@edumail.at (E.F.); fiorella.schischlik@nih.gov (F.S.); dalpar@cemm.oeaw.ac.at (D.A.)

⁶ Clinical Institute of Pathology, Medical University of Vienna, 1090 Vienna, Austria; ana-iris.schiefer@meduniwien.ac.at (A.I.S.); ingrid.simonitsch-klupp@meduniwien.ac.at (I.-S.-K.)

* Correspondence: klaus.geissler@wienkav; Tel.: +43-01-80110-3122; Fax: +43-01-80110-2671

Supplementary

Table S1. Circulating CFU-GM and BFU-E numbers, CFU-GM/BFU-E ratio and driver mutation status in patients with myelofibrosis.

Pat Nr	Sex	Age	Driver Mutation Status	Follow up Time in Months	CFU-GM/ml	BFU-E/ml	CFU-GM/BFU-E Ratio
1	m	71	JAK2 wt	12	222	24737	0.01
2	m	73	JAK2 mut	7	81	1013	0.08
3	m	48	JAK2 mut	43	134	1562	0.09
4	f	74	JAK2 mut	23	73	640	0.11
5	f	50	JAK2 mut	33	473	4476	0.11
6	m	60	JAK2 mut	63	1533	12209	0.13
7	m	75	JAK2 mut	21	196	1500	0.13
8	m	67	JAK2 mut	40	332	2363	0.14
9	f	50	JAK2 mut	7	301	2040	0.15
10	m	72	JAK2 mut	20	192	1267	0.15
11	f	44	JAK2 mut	32	586	3320	0.18
12	m	71	JAK2 mut	52	836	4251	0.2
13	f	68	JAK2 mut	71	320	1469	0.22
14	f	45	JAK2 mut	29	1114	4129	0.27
15	m	69	CALR mut	10	1364	5072	0.27
16	f	58	Triple neg	44	373	1325	0.28
17	f	72	JAK2 wt	23	6938	24877	0.28
18	f	45	JAK2 mut	14	448	1627	0.28
19	m	52	CALR mut	6	1152	4018	0.29
20	f	82	CALR mut	76	563	1809	0.31
21	f	88	JAK2 mut	50	420	1370	0.31
22	f	58	JAK2 mut	51	1803	5900	0.31
23	f	53	JAK2 mut	21	513	1665	0.31

24	m	46	JAK2 mut	120	538	1746	0.31
25	f	35	CALR mut	41	1663	5088	0.33
26	m	25	CALR mut	43	2822	8248	0.34
27	m	67	Triple neg	0	154	450	0.34
28	f	64	JAK2 mut	7	989	2748	0.36
29	m	49	CALR mut	62	3082	8386	0.37
30	f	54	JAK2 mut	72	2022	5184	0.39
31	f	75	CALR mut	3	1734	4167	0.42
32	f	78	JAK2 mut	166	747	1737	0.43
33	f	45	JAK2 wt	55	910	1918	0.47
34	f	64	JAK2 mut	66	478	1014	0.47
35	m	46	JAK2 wt	24	2876	5941	0.48
36	m	36	JAK2 mut	83	47	94	0.5
37	m	69	JAK2 mut	40	2251	4480	0.50
38	m	82	CALR mut	36	3349	6658	0.50
39	m	70	JAK2 mut	54	3214	6022	0.53
40	f	66	MPL mut	83	1767	3304	0.53
41	f	41	CALR mut	66	1395	2545	0.55
42	f	76	JAK2 mut	51	784	1395	0.56
43	m	61	CALR mut	50	2449	4194	0.58
44	f	66	CALR mut	138	1239	2145	0.58
45	m	55	CALR mut	7	4045	6849	0.59
46	m	55	JAK2 mut	139	875	1416	0.62
47	m	63	CALR mut	72	701	1069	0.66
48	f	82	JAK2 mut	80	2570	3674	0.70
49	m	51	JAK2 mut	148	6349	8997	0.71
50	m	73	JAK2 mut	68	1103	1538	0.72
51	f	78	CALR mut	47	576	797	0.72
52	m	70	JAK2 mut	35	1682	2181	0.77
53	f	52	CALR mut	4	785	1009	0.78
54	f	86	Triple neg	10	28761	34776	0.82
55	m	57	JAK2 mut	123	6754	7085	0.95
56	m	62	JAK2 mut	30	10328	8977	1.15
57	f	42	CALR mut	28	1681	1225	1.37
58	f	75	JAK2 mut	83	4034	2800	1.44
59	f	74	JAK2 mut	53	4840	2728	1.77
60	m	58	JAK2 mut	81	9375	5239	1.79
61	f	68	JAK2 mut	46	3723	1887	1.97
62	f	71	JAK2 mut	23	4096	1946	2.1
63	m	73	JAK2 mut	5	61923	28897	2.14
64	m	72	JAK2 wt	14	5500	2200	2.5
65	f	53	JAK2 mut	62	1706	552	3.09
66	m	52	CALR mut	147	10639	3224	3.30
67	m	60	MPL mut	139	3270	688	4.75
68	f	68	CALR mut	40	13231	2623	5.04
69	f	71	JAK2 mut	18	4770	875	5.45
70	m	55	JAK2 mut	16	1026	134	7.66
71	m	86	CALR mut	10	22418	2842	7.89
72	m	75	JAK2 mut	1	12242	1243	9.85
73	m	71	JAK2 mut	65	98344	5887	16.70
74	m	61	MPL mut	55	17758	539	32.94
75	m	78	JAK2 mut	35	7700	172	44.76
76	m	70	JAK2 wt	2	53033	851	62.32
77	m	59	JAK2 mut	38	4000	57	70.18
78	m	58	CALR mut	3	16524	229	72.16
79	m	81	JAK2 wt	20	9000	95	94.73
80	m	58	Triple neg	47	11260	97	116.08
81	m	81	JAK2 mut	22	2456	11	223.27

Mut, mutated; wt, wildtype; NA, not available; CFU-GM, colony-forming unit granulocyte/macrophage; BFU-E, burst-forming unit erythroid.

Table S2. Multivariate Cox regression analysis of overall survival.

Parameter	Hazard Ratio	95% Confidence Interval	P-Value
Skewing present	3.31	1.22-8.96	0.019
WBC >25 × 10 ⁹ /L	4.50	0.93-21.82	0.062
Hb <10 g/dL	2.75	0.92-8.27	0.071
PLT <100 × 10 ⁹ /L	3.39	1.24-9.30	0.018
PB Blasts present	1.55	0.6-3.97	0.363
Age >65 years	3.98	1.96-10.85	0.007

WBC, white blood cell count; Hb, hemoglobin; PLT, platelet count, PB, peripheral blood; The proportional hazards model was used to evaluate the prognostic importance of each factor while simultaneously considering the effects of other covariates. For the purpose of clinical utility, continuous covariates were regarded as dichotomous, with categories determined based on consideration of previously reported cut off points in this disease. Covariates including skewing, WBC, Hb, PLT, PB blasts and age, respectively, have been used to make the adjustments.

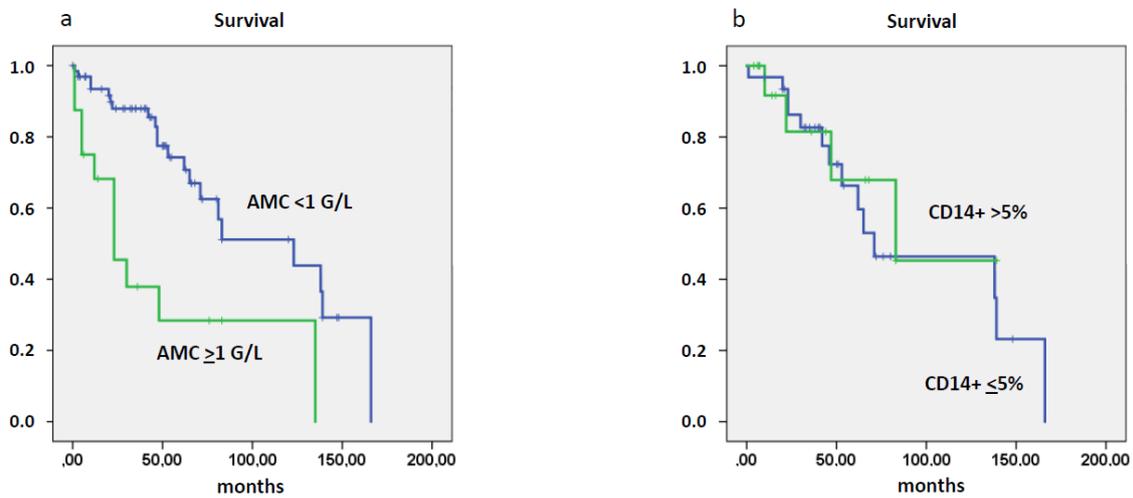


Figure S1. Overall survival of myelofibrosis patients stratified by the presence or absence of absolute monocytosis ≥ 1 G/L (**a**) and the proportion of CD14 positive bone marrow cells $> 5\%$ (**b**). AMC, absolute monocyte count; Figure S1a: median overall survival in patients with AMC ≥ 1 G/L 23 months vs. 83 months in patients with AMC < 1 G/L ($p = 0.006$). Figure S1b: median overall survival in patients with CD14 pos cells $> 5\%$ 83 months vs. 65 months in patients with CD14 pos cells $\leq 5\%$ ($p = 0.566$).