Targeting Mutated BRAF with Vemurafenib is Safe and Highly Active in Relapsed/Refractory Hairy Cell Leukemia: a Phase-2 Italian Clinical Trial (HCL-PG01)

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BACKGROUND. Purine analogues (PA) are effective in hairy cell leukemia (HCL), but ~40% of patients relapse and respond progressively less well to these drugs, which can also cause cumulative myelotoxicity. Our discovery of the BRAF-V600E kinase-activating mutation as the genetic lesion underlying HCL opens the way to a targeted therapy with BRAF inhibitors.

METHODS. We completed the first clinical trial with the BRAF inhibitor vemurafenib in refractory/relapsed HCL. This is a phase-2, academic, single-arm, Italian, multicenter study (EudraCT 2011-005487-13). In 11 months, 8 centers enrolled 28 patients requiring treatment due to cytopenias (**TABLE 1**), who were refractory to (n=6) or early relapsed after (n=21) a PA, or had severe toxicity from a PA (n=1). Previous treatments also included interferon, rituximab and splenectomy in 13, 14 and 8 patients, respectively.

RESULTS. At the oral dose of 960 mg twice daily for a median of 16 weeks, vemurafenib was overall well tolerated. The most frequent drug-related adverse events were arthralgias, skin toxicities and pancreatitis, usually of grade 1-2. Vemurafenib was not myelotoxic. We observed no cutaneous squamous cell carcinomas/keratoachantomas (as frequently reported in BRAF-V600E+ melanoma patients treated with vemurafenib), but 2 patients developed skin basaliomas and 1 patient a cutaneous superficial melanoma, all treated with simple excision.

Interestingly, *in vivo* exposure to vemurafenib caused decreased expression of the HCL marker CD25 in 15/19 (79%) evaluable patients (**FIGURE 1A** – bone marrow flow cytometry gated on CD45+ cells before and after 7-14 days of vemurafenib), as well as smoothening of the hairy surface in a patient unusually featuring a leukemic lymphocytosis easy to monitor morphologically before and after 2-3 days of vemurafenib (**FIGURE 1B**; left: blood smear; right: confocal microscopy of purified HCL cells after green labeling by phalloidin of the F-actin rich hairy projections).

In 26 evaluable patients, the overall response rate was a striking 96%: 9/26 (34.6%) complete remissions (CRs) and 16/26 (61.4%) partial remissions (PRs), obtained after a median of 8 weeks (**TABLE 1**). With a median follow-up of 23 months post-treatment, the median relapse-free and treatment-free survivals were 19 and 25 months respectively in CR patients, and 6 and 18 months respectively in PR patients (**FIGURE 2**).

Bone marrow immunohistochemistry showed residual HCL cells in all patients, even those in CR (**FIGURE 3**; left panel: 75% HCL cells before therapy; right panel: 5-10% scattered HCL cells after 8 weeks of vemurafenib, not visible by morphological stains and thus complying with a CR). Residual HCL cells exhibited persistent phospho-ERK (pERK) expression at the end of treatment in 6/13 evaluable cases (**FIGURE 4** - leukemic cells are labeled by the B-cell marker PAX5; PAX5-negative bone marrow stromal cells act as internal positive control for the blue pERK staining in the right panel). The persistence of pERK+ HCL cells at the end of treatment correlated with a shorter progression-free survival (median of 7.8 vs 13 months; p=0.004), and suggests by-pass reactivation of MEK/ERK as a resistance mechanism to vemurafenib in at least some patients.

Four CR patients and 6 PR patients, who respectively relapsed 9-18 months and 5-14 months post-treatment, received a second short course of vemurafenib for 4-12 weeks. The 3/4 evaluable CR patients obtained a second CR (n=2) and a PR (n=1); response duration could be evaluated in 2/3 patients, one in CR and one in PR, and was respectively 7 and 2 months after retreatment as compared to 12 and 5 months after first treatment. In the 5/6 evaluable PR patients retreated with vemurafenib, we observed 1 second PR (lasting 3 months) and 4 minor responses.

CONCLUSIONS. A brief oral treatment with vemurafenib was safe, and proved highly and rapidly active in heavily pre-treated HCL patients. Retreatment with vemurafenib was more effective in patients relapsing after CR than after PR.

Table 1. Patients' features and response to vemurafenib

				Blood counts and spleen size								
				Neut.	PLT	Hb	Spleen°	Response to	Weeks of treatment		Relapse-free survival	Treatment-free survival
Pt.	Sex	Age	Previous lines of therapy	/mm³	x10³/mm³	g/dl	cm	Vemurafenib	until best response	total	(in months¶)	(in months¶)
1	M	44	DCF, IFN, CDA, IFN, RTX, IFN	596	39	13.1	18	PR	8	20	6	7
2	M	76	DCF, DCF, CDA	399	97	9.8	13	CR	8	20	12	18
3	M	58	IFN, RTX, CDA	480	62	11	18	PR	12	20	3	8
4	F	52	Spl., 2CDA*, DCF+RTX	1443	139	10	-	PR	12	16	7	8
5	F	45	CDA*	1050	140	9.8	≤12	PR	16	16	21+	24+
6	F	81	DCF^	560	76	12.6	16	PR	12	16	15	21+
7	M	27	CDA*, Spl., RTX	1363	177	14.5	-	PR	8	16	6	11
8	M	77	Spl., IFN, CDA, CDA, IFN, CDA, RTX, RTX	179	36	8.9	-	CR	8	8	5	9
9	M	57	IFN, IFN, DCF, CDA, CDA, IFN, DCF+RTX	480	73	8.6	25	MR	16	16	/	4
10	M	68	Spl., CDA, DCF, CDA, IFN, CDA	290	6	8.2	-	PR§	12	12	14	18
11	M	49	DCF, CDA, CDA, RTX, IFN, CDA	861	66	15.3	25	CR	12	12	9	25
12	M	57	CDA, CDA, CDA, DCF	644	70	12.7	25	PR	4	16	3	24+
13	M	71	DCF, 2CDA, RTX, DCF, RTX, CDA	830	51	11.9	≤12	CR	8	8	21+	25+
14	M	70	Spl., IFN, CDA, RTX	710	47	9.2	-	PR	8	16	5	5
15	M	80	CDA+RTX, RTX, Spl., CDA	5710	85	15.4	-	not evaluable"				
16	M	84	IFN, DCF, CDA, CDA, IFN	1123	79	11.2	≤12	CR	4	8	23+	23+
17	M	50	CDA, CDA	966	94	13.4	13.5	PR	16	16	3	18+
18	M	43	CDA, RTX	985	30	15.2	18	PR	4	16	1	13
19	M	52	CDA*, IFN	1160	66	13.9	≤12	PR	4	16	3	21+
20	M	51	CDA, CDA	1336	71	13.5	16	PR	8	16	9	14
21	M	38	CDA, CDA	628	48	17	13.5	CR	4	8	24+	24+
22	M	67	IFN, CDA, CDA+RTX	749	56	15.3	17	CR	8	8	25+	25+
23	F	39	DCF*	213	23	9.2	17.5	CR	4	8	12	18
24	M	39	CDA	1363	113	14.8	17	CR	5	8	19	23+
25	M	57	IFN, CDA, CDA, CDA, IFN	2310	49	10.5	≤12	PR§	8	16	10	20+
26	M	68	Spl., IFN, IFN, IFN, DCF, DCF, IFN, CDA, RTX, CDA, CDA, CDA	1300	120	10	-	PR	10	14	8	18
27	M	72	CDA, CDA+RTX	1664	306	9.7	≤12	not evaluable"				
28	M	56	IFN, CDA*, DCF, RTX, Spl.	425	304	14.5	-	PR	8	14	3	18+
			Median	846	71	12.2	16	n.a.	8	16	9	21.5

Neut.: neutrophils; PLT: platelets; Hb: hemoglobin; DCF: pentostatin; IFN: interferon; CDA: cladribine; RTX: Rituximab; Spl.: splenectomy; n.a.: not applicable

*Primary refractory

^Severe septic arthritis after DCF

°Longest diameter

§With delayed platelet recovery

"Off-study after ≤1 week of therapy for drug-unrelated acute myocardial infarction (pt. 15) or for consent withdrawal due to drug-related, reversible, grade-3 pancreatitis (pt. 27)

¶From the end of treatment

Figure 1

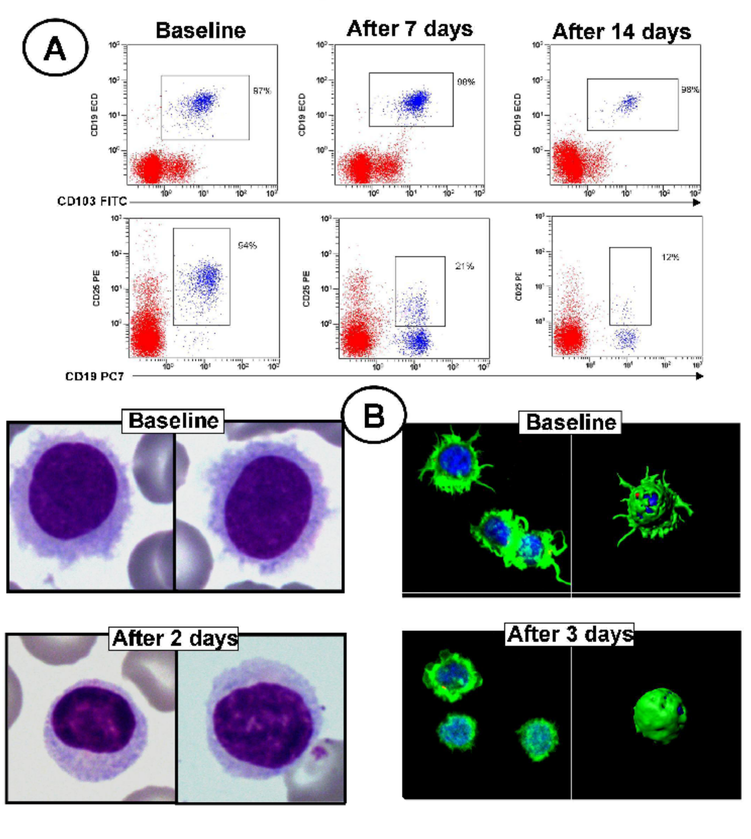


Figure 2

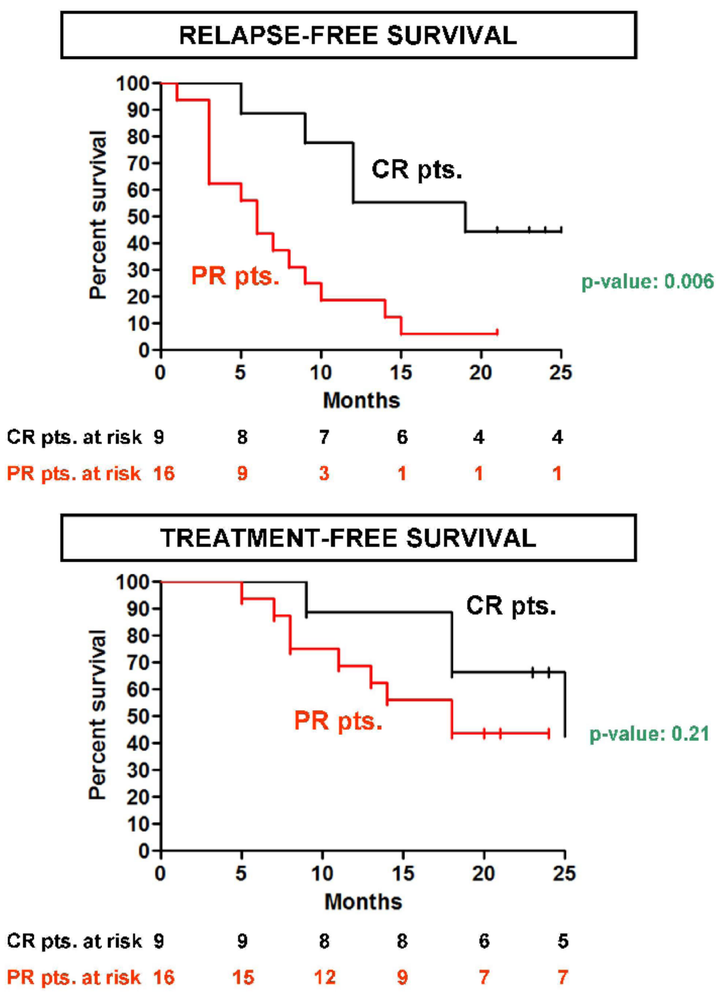
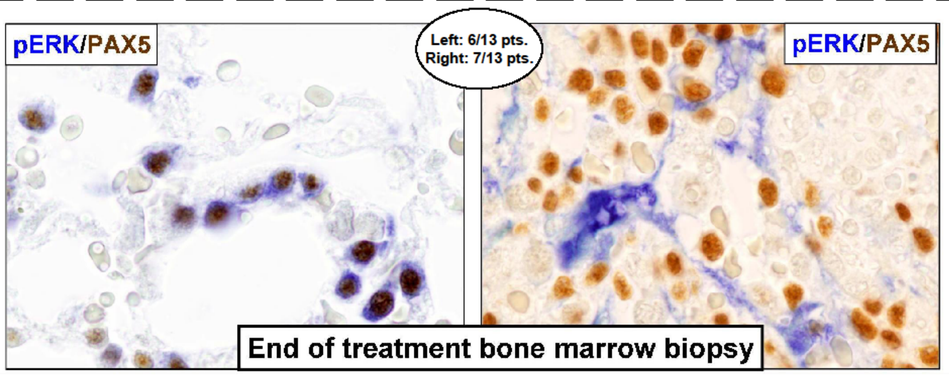


Figure 3

Figure 4



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