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LETTER TO THE EDITOR

The predictive value of *human organic cation transporter 1* and *ABCB1* expression levels in different cell populations of patients with de novo chronic myelogenous leukemia

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The role of imatinib (IMA) intracellular transport in overall resistance and treatment outcome prediction has been intensively studied since the first description of the main influx (hOCT-1) and efflux (ABCB1) transport proteins [1]. Several studies that correlate the pretreatment activity/mRNA expression of IMA transporters with subsequent therapy response have been reported to date [2–5]. However, different cell populations from patients who received various degrees of pretreatment were used for these analyses. Recent data have shown that the composition of the analyzed material at the time of testing (i.e., percentage of different cell types) has a critical impact on the resultant activity/mRNA expression of IMA transporters, and thus on the overall correlation and data interpretation [6–9]. Therefore, it is not surprising that the predictive value of pretreatment transporter characteristics remains controversial.

In this study, we investigated the predictive value of pretreatment mRNA expression levels of *hOCT-1* and *ABCB1* in different cell populations with regard to the response to therapy at 6 and 12 months of IMA therapy. Expression levels were assessed in peripheral blood (PB) leukocytes (LEU, $n = 30$), polymorphonuclear cells (PMNC, $n = 23$), and mononuclear cells (MNC, $n = 21$) of PB LEU obtained from 30 patients (Table 1) with de novo chronic myelogenous leukemia (CML). Moreover, the available bone marrow cells (BM) were also included and analyzed (BM, $n = 11$). The PB and BM samples were obtained, processed, and analyzed as previously described [6]. Responses to therapy were classified according the European LeukemiaNet 2009 (ELN) criteria [10]: responders show an optimal response at 6 months and 12 months, while non-responders reflect a suboptimal response or therapy failure. The assessed pretreatment expression levels were stratified into two groups according to the median—a low-mRNA expression group below the median and a high-mRNA expression group equal to or above the median. The statistical evaluation of the data obtained was performed using the Fisher's exact tests and summarized in Table 2.

1 *hOCT-1*

The results obtained clearly indicate that there was no statistically significant relationship between pretreatment mRNA expression levels assessed in any selected cell populations and response to therapy at 6 and 12 months (all with $p > 0.05$). Moreover, at both monitored time points, there were a substantial proportion of patients who

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Table 1 Patient characteristics: white blood cell (WBC) count (10^9/L); age at diagnosis (years); Sex M (male), F (female); initial IMA dosage (mg/day), optimized IMA dosage (mg/day); Sokal score; Hasford score; therapy responses at 6 and 12 months of IMA therapy according the ELN 2009 criteria [10]

Patient No.	WBC (10^9/L)	Age	Sex	Initial IMA dosage (mg/day)	Optimized IMA dosage (mg/day)	Sokal score	Hasford score	ELN response at 6 months	ELN response at 12 months
1	173.0	53	F	400	400	1.53	1543.8	Optimal	Optimal
2	31.9	56	M	400	350	0.71	1077.0	Optimal	Optimal
3	49.0	59	F	400	200	0.89	1176.7	Optimal	Optimal
4	350.0	19	M	400	250	0.57	696.6	Optimal	N/A
5	41.9	69	F	400	300	0.84	953.1	Optimal	Optimal
6	287.0	66	F	400	400	1.09	1370.3	Optimal	Optimal
7	113.9	66	F	400	400	1.59	1495.0	Suboptimal + failure	Suboptima + failure
8	10.3	77	M	400	300	2.78	1957.0	Optimal	Suboptima + failure
9	152.0	75	M	400	400	1.25	1071.3	Optimal	Optimal
10	37.1	67	M	400	400	0.86	666.6	Optimal	Optimal
11	13.8	30	M	400	400	0.58	534.3	Optimal	Optimal
12	111.0	41	F	400	200	1.86	1671.2	Suboptimal + failure	N/A
13	20.5	41	F	400	350	0.61	82.6	Optimal	Optimal
14	36.5	66	F	400	400	1.06	1054.2	Optimal	Optimal
15	32.6	44	M	400	400	1.20	846.9	Optimal	Optimal
16	134.0	37	F	400	300	0.75	403.3	Optimal	Suboptima + failure
17	6.3	68	F	400	250	1.01	1135.4	Optimal	Suboptima + failure
18	66.8	71	M	400	250	0.78	707.0	Suboptimal + failure	N/A
19	8.0	76	F	400	400	2.95	1245.1	Optimal	Optimal
20	11.3	71	F	400	400	1.60	1379.6	Optimal	Optimal
21	23.2	46	F	400	400	0.66	451.7	Suboptimal + failure	N/A
22	60.9	67	M	400	400	0.88	666.6	Optimal	Optimal
23	68.0	49	M	400	400	0.82	579.8	Optimal	Optimal
24	48.5	66	M	400	400	0.94	1035.7	Optimal	Optimal
25	247.0	65	M	400	400	1.51	1396.3	Optimal	Optimal
26	15.2	58	M	300	400	0.80	1035.7	Optimal	Optimal
27	94.1	59	M	400	350	0.98	928.0	Optimal	Optimal
28	152.0	31	F	400	350	0.78	660.3	Optimal	Optimal
29	55.7	69	M	400	400	1.12	850.3	Optimal	Optimal
30	70.4	61	M	400	400	1.50	958.6	Optimal	Optimal

exhibited an optimal response regardless of low pretreatment *hOCT-1* mRNA expression, which suggests that the therapy response at these time points is not directly related to these values. Similar results were obtained when non-responders were evaluated.

2 *ABCB1*

An analogous evaluation was performed for pretreatment *ABCB1* mRNA expression levels. Similarly, no statistically significant relationship between the pretreatment *ABCB1* values and therapy response was observed (Table 2).

In summary, our findings show no statistically significant relationship between the pretreatment levels of *hOCT-1* or *ABCB1* mRNA expression in different cell populations and therapy response at 6 and 12 months of IMA therapy. Despite the short observational period and small cohort of patients, our data indicate that there are a substantial proportion of patients who exhibit an optimal response regardless of their low pretreatment *hOCT-1* mRNA expression or high *ABCB1* expression, and vice versa (i.e., suboptimal response/therapy failure regardless of high *hOCT-1* or low *ABCB1* expression). Similar results were reported in a larger study, where substantial frequencies of optimal responses were also associated with low hOCT-1 pretreatment values [5], indicating some analogy between the assessment of mRNA expression and functional activity of these transport proteins and their predictive values. Regardless of the fact that some correlation and similarities

Table 2 Pretreatment mRNA expression levels of *hOCT-1* and *ABCB1* in de novo CML patients and therapy responses at 6 and 12 months of IMA therapy according the ELN 2009 criteria [10]

Transporter	Cell type	mRNA expression [median (%)]	Group	ELN response at 6 months		*p* value	ELN response at 12 months		*p* value
				Optimal (%)	Suboptimal + failure (%)		Optimal (%)	Suboptimal + failure (%)	
hOCT-1	LEU	15.80	Low	12 (80.0)	3 (20.0)	0.589	11 (91.7)	1 (8.3)	0.598
			High	14 (93.3)	1 (6.7)		11 (78.6)	3 (21.4)	
	PMNC	78.59	Low	9 (81.8)	2 (18.2)	0.217	8 (80.0)	2 (20.0)	1.000
			High	12 (100.0)	0 (0.0)		9 (81.8)	2 (18.2)	
	MNC	0.93	Low	8 (88.9)	1 (11.1)	1.000	6 (85.7)	1 (14.3)	1.000
			High	11 (91.7)	1 (8.3)		9 (75.0)	3 (25.0)	
	BM	2.71	Low	5 (100.0)	0 (0.0)	1.000	5 (100.0)	0 (0.0)	1.000
			High	6 (100.0)	0 (0.0)		5 (83.3)	1 (16.7)	
ABCB1	LEU	5.18	Low	13 (81.3)	3 (18.7)	0.602	12 (85.7)	2 (14.3)	1.000
			High	13 (92.9)	1 (7.1)		10 (83.3)	2 (16.7)	
	PMNC	1.86	Low	7 (77.8)	2 (22.2)	0.156	6 (75.0)	2 (25.0)	1.000
			High	13 (100.0)	0 (0.0)		10 (83.3)	2 (16.7)	
	MNC	10.96	Low	9 (81.8)	2 (18.2)	0.476	7 (77.8)	2 (22.2)	1.000
			High	10 (100.0)	0 (0.0)		8 (80.0)	2 (20.0)	
	BM	2.99	Low	6 (100.0)	0 (0.0)	1.000	5 (83.3)	1 (16.7)	1.000
			High	5 (100.0)	0 (0.0)		5 (100.0)	0 (0.0)	

The mRNA expression levels were calculated using the following formula: $gene^{(\text{absolute copies})}/GUS^{(\text{absolute copies})} \times 100\%$ [6, 7] and stratified into groups according to the median—a low-mRNA expression group below the median, and a high-mRNA expression group equal to or above the median

between these two independent approaches have been reported [2, 6, 9], it must be noted here that this does not necessarily indicate that there is a correlation between the predictive values of expression of transport proteins and their activity.

In conclusion, our data focused on the predictive value of mRNA expression of transport proteins, clearly indicate that assessment of pretreatment *hOCT-1* and *ABCB1* mRNA expression is probably not suitable for implementation in routine clinical practice. The effect of individual cell types, tumor burden dependence, and other pre-analytical and analytical biases [6, 7] lead to the conclusion that the desired stratification of CML patients (using pretreatment *hOCT-1* and *ABCB1* mRNA levels) into responders/non-responders prior to IMA therapy is difficult to obtain. Therefore, we emphasize that complex predictors (i.e., Sokal or Euro score), even in the era of tyrosine kinase inhibitors, are still better tools for predicting treatment outcome than pretreatment transporter mRNA level.

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Conflict of interest All authors have nothing to disclose.

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