Single nucleotide polymorphisms in *ABCB1* and *CBR1* can predict toxicity to R-CHOP type regimens in patients with diffuse non-Hodgkin lymphoma

The prediction of high-grade toxicities in cancer patients would clearly help their overall management by intensifying the surveillance and monitoring specific parameters in patients at risk. We studied the relation between selected genetic polymorphisms and treatment-associated grade 3 or higher toxicities in 760 patients with diffuse large B-cell lymphoma receiving R-CHOP or R-ACVBP, and found significant correlation between rs2229109 in *ABCB1* and vomiting (P=0.003) and diarrhea (P=0.007), and between rs20572 and rs9024 in *CBR1* and anemia, thrombocytopenia and diarrhea (approx. P=0.02). These results suggest that genotyping of peripheral blood cells could help predict severe toxicity in patients receiving R-CHOP type regimens.

Both functional and non-functional polymorphisms have been shown to be correlated to the toxicity of R-CHOP-based treatment of non-Hodgkin lymphoma (NHL).[1,2] In order to confirm such correlations, we studied 14 polymorphisms in 11 genes previously shown to be involved in the metabolism and cellular response to drugs used in this treatment (*Online Supplementary Table S1*). Genotypes were determined as described before[3] or by TaqMan SNP Genotyping Assays (Life Technologies, Carlsbad, CA, USA) on blood samples from 760 patients out of a total of 1703 patients included in prospective randomized trials (*clinicaltrials.gov identifier: 00140660, 00140595, 00144807, 00169143, 00144755, 0187424* and *00135499*) organized by the LYSA, and correlated with grade 3 toxicities prospectively evaluated for each cycle using the NCI CTC V3 scale.

Patients' characteristics are listed in Table 1. Initial analyses on the occurrence and distribution of toxicities showed that (R)-ACVBP was associated with a significantly (P<0.001) higher incidence of toxicity as compared to R-CHOP, with a higher frequency of anemia (48.0% *vs.* 24.0%), thrombocytopenia (50.5% *vs.* 19.3%), febrile neutropenia (50.8% *vs.* 22.2%), and mucositis (26.6% *vs.* 7.7%), but not for diarrhea and vomiting or for delay in treatment administration (Table 2). The call rate for each polymorphism was 99.5%-100% except for rs2740574 in CYP3A4 (96.4%), and their distribution was consistent with Hardy-Weinberg equilibrium except for GSTP1 (P=0.003).

As the toxicity profile differed between R-CHOP and R-ACVBP, we first analyzed for correlation with toxicity in the two treatment groups ((R)-ACVBP and R-CHOP) separately. Here, we observed correlations for febrile neutropenia (P=0.039), and vomiting (P=0.043) with rs2229109 in *ABCB1*, and for febrile neutropenia with rs1695 (P=0.030) in *GSTP1* in (R)-ACVBP-treated patients. In R-CHOP-treated patients, we observed correlations for diarrhea (P=0.041), vomiting (P=0.031), and mucositis (P=0.004) with rs2229109 in ABCB1, for febrile neutropenia (P=0.031), and treatment delay (P=0.040) with rs20572 in *CBR1*, for febrile neutropenia with rs9024 (P=0.044) in *CBR1*, and for treatment delay with rs714368 (P=0.015) in SLC22A16.

In a second series of analyses, we searched for correlations between grade 3 or higher toxicities and all genotypes in the complete cohort (Table 3). SNP in *ABCB1* and both SNP in *CBR1* correlated with the occurrence of grade 3-4 toxicities. rs2229109 (Ser400Asn) in *ABCB1* was associated with increased risk of high-grade diarrhea (P=0.007) and

Table 1. Clinical and biochemical characteristics of patients included in the current study.

	N=760		N=1703	
Histological subtype				
DLBCL	586	(77.1%)	1217	(71.5%)
Other	98	(12.9%)	322	(18.9%)
Unknown or insufficient sample	76	(10%)	164	(9.6 %)
Age (years)				
Mean (STD)	58.4 (16.49)		58.8	
Median	59.0		61.0	
Min / max	18 / 93		18 / 95	
Age in classes				
≤ 60 years	399	(52.5%)	837	(49.1%)
> 60 years	361	(47.5%)	866	(50.9%)
Sex				
Male	441	(58.0%)	939	(55.1%)
Female	319	(42.0%)	764	(44.9%)
IPI				
0	115	(15.1%)	214	(12.6%)
1	142	(18.7%)	331	(19.4%)
2	172	(22.6%)	384	(22.5%)
3	174	(22.9%)	387	(22.7%)
4	111	(14.6%)	280	(16.4%)
5	46	(6.1%)	107	(6.3%)
Age-adjusted IPI				
0	140	(18.4%)	266	(15.6%)
1	280	(36.8%)	657	(38.6%)
2	256	(33.7%)	579	(34.0%)
3	84	(11.1%)	201	(11.8%)
Ann Arbor stage				
Stage 1	107	(14.1%)	224	(13.2%)
Stage 2	129	(17.0%)	305	(17.9%)
Stage 3	116	(15.3%)	253	(14.9%)
Stage 4	408	(53.7%)	921	(54.1%)
Performance status (ECOG)				
0	343	(45.2%)	762	(44.8%)
1	297	(39.1%)	671	(39.4%)
2	108	(14.2%)	244	(14.3%)
3	11	(1.4%)	24	(1.4%)
Unknown	1		1	
LDH				
≤Normal	359	(47.2%)	728	(42.7%)
>Normal	401	(52.8%)	975	(57.3%)
Number of extranodal sites				
≤1	483	(63.6%)	1073	(63.0%)
>1	277	(36.4%)	630	(37.0%)
Bone marrow biopsy				
Not involved	581	(76.4%)	1287	(75.7%)
Involved	115	(15.1%)	269	(15.8%)
Unknown	64	(8.5%)	147	(8.6%)
Mass > 10 cm				
No	612	(81.2%)	1251	(80.1%)
Yes	142	(18.8%)	311	(19.9%)
Unknown	6		141	
B symptoms				
No	497	(65.6%)	1110	(65.3%)
Yes	261	(34.4%)	590	(34.7%)
Unknown	2		3	
β^2 microglobulin				
< 3 mg/L	448	(68.7%)	940	(66.6%)
≥ 3 mg/L	204	(31.3%)	472	(33.4%)
Unknown	108		291	
Albumin				
≤ 35 g/L	190	(27.7%)	446	(29.7%)
> 35 g/L	495	(72.3%)	1057	(70.3%)
Unknown	75			200

DLBCL: diffuse large B-cell lymphoma; IPI: International Prognostic Index; LDH: lactate dehydrogenase.

Table 2. Comparison of grade ≥3 toxicity and delay in treatment in patients treated with R-CHOP or (R)-ACVBP.

Toxicity parameter	Occurrence	All (n=760)		R-CHOP (n=441)		(R)-ACVBP (n=319)		χ^2 *P*
Anemia	No	501	(65.9%)	335	(76.0%)	166	(52.0%)	<0.001
	Yes	259	(34.1%)	106	(24.0%)	153	(48.0%)	
Thrombocytopenia	No	514	(67.6%)	356	(80.7%)	158	(49.5%)	<0.001
	Yes	246	(32.4%)	85	(19.3%)	161	(50.5%)	
Febrile neutropenia	No	500	(65.8%)	343	(77.8%)	157	(49.2%)	<0.001
	Yes	260	(34.2%)	98	(22.2%)	162	(50.8%)	
Diarrhea	No	733	(96.4%)	421	(95.5%)	312	(97.8%)	0.085
	Yes	27	(3.6%)	20	(4.5%)	7	(2.2%)	
Vomiting	No	729	(95.9%)	422	(95.7%)	307	(96.2%)	0.707
	Yes	31	(4.1%)	19	(4.3%)	12	(3.8%)	
Mucositis	No	641	(84.3%)	407	(92.3%)	234	(73.4%)	<0.001
	Yes	119	(15.7%)	34	(7.7%)	85	(26.6%)	
Treatment delay	No	551	(72.5%)	331	(75.1%)	220	(69.0%)	0.063
	Yes	209	(27.5%)	110	(24.9%)	99	(31.0%)	

Table 3. Statistically significant correlations between grade ≥3 toxicities and genotypes in all patients comparing common homozygotes (HH) with heterozygotes (Hh) and rare homozygotes (hh). Correlations were performed with the Cochran Mantel Haenszel test with stratification on type of treatment [(R-CHOP or (R)-ACVBP)]. Other studied polymorphisms were rs172378 in *C1QA*, rs1001179 and rs10836235 in *CAT*, rs2740574 in *CYP3A4*, rs1695 in *GSTP1*, rs12210538 and rs714368 in *SLC22A10*, rs4880 in *SOD2*, rs8175347 in *UGT1A1* and copy number variations for *GSTM1* and *GSTT1*.

Gene	SNP	Toxicity parameter	Occurrence	HH[a]		Hh[a]		hh[a]		All		CMH *P*
		Diarrhea	No	676	(97.0%)	57	(90.5%)	–	–	733	(96.4%)	0.007
			Yes	21	(3.0%)	6	(9.5%)	–	–	27	(3.6%)	
ABCB1	rs2229109	Vomiting	No	673	(96.6%)	56	(88.9%)	–	–	729	(95.9%)	0.003
			Yes	24	(3.4%)	7	(11.1%)	–	–	31	(4.1%)	
		Anemia	No	417	(67.7%)	81	(60.4%)	3	(30.0%)	501	(65.9%)	0.018
			Yes	199	(32.3%)	53	(39.6%)	7	(70.0%)	259	(34.1%)	
CBR1	rs20572	Thrombocytopenia	No	426	(69.2%)	85	(63.4%)	3	(30.0%)	514	(67.6%)	0.015
			Yes	190	(30.8%)	49	(36.6%)	7	(70.0%)	246	(32.4%)	
		Diarrhea	No	595	(96.6%)	130	(97.0%)	8	(80.0%)	733	(96.4%)	0.019
			Yes	21	(3.4%)	4	(3.0%)	2	(20.0%)	27	(3.6%)	
		Anemia	No	417	(67.5%)	81	(61.4%)	3	(30.0%)	501	(65.9%)	0.026
			Yes	201	(32.5%)	51	(38.6%)	7	(70.0%)	259	(34.1%)	
CBR1	rs9024	Thrombopenia	No	427	(69.1%)	84	(63.6%)	3	(30.0%)	514	(67.6%)	0.017
			Yes	191	(30.9%)	48	(36.4%)	7	(70.0%)	246	(32.4%)	
		Diarrhea	No	597	(96.6%)	128	(97.0%)	8	(80.0%)	733	(96.4%)	0.019
			Yes	21	(3.4%)	4	(3.0%)	2	(20.0%)	27	(3.6%)	

HH[a]: common homozygous; Hh[a]: heterozygous; hh[a]: rare homozygous.

vomiting (*P*=0.003) in patients with CT genotype, whereas there were no patients with TT genotype for this SNP. The rare homozygous genotypes of the two silent SNP in CBR1 (Ala209Ala and 3'-UTR) were found to be associated with higher incidence of grade 3-4 anemia (*P*=0.018 and 0.026, respectively), thrombocytopenia (*P*=0.015 and 0.017), and diarrhea (*P*=0.019).

Results presented here suggest that polymorphisms in *ABCB1* (coding for the ATP binding cassette efflux protein Pgp) and *CBR1* (coding for carbonyl reductase 1) could be used to identify NHL patients with a high risk of myeloid or digestive toxicity after treatment with R-CHOP-type regimens. Both of these genes have been reported to be involved in anthracycline transport or metabolism, indicating a possible mechanistic role in the correlation.[4]

ABCB1 is well described as a membrane transporter of anthracyclines, as well as other hydrophobic drugs used in the treatment of NHL patients, such as etoposide or vinca alkaloids.[5] Several studies have correlated ABCB1 polymorphisms with sensitivity to therapeutic compounds. Interestingly, Yao *et al.* reported on a set of tag SNP in *ABCB1* and did not observe any correlation to grade 3 or higher hematologic or intestinal toxicity in cyclophosphamide- and doxorubicin-treated breast cancer patients.[6] However, these tag SNP did not cover the position of rs2229109 that was shown to be correlated in our study. This discrepancy could be due to the fact that rs2229109 is a non-synonymous polymorphism, and that the two protein variants potentially do not have the same activity.

Carbonyl reductase 1 (*CBR1*) is an anthracycline-metabolizing enzyme. It was reported that increased expression of CBR1 was associated with reduced sensitivity of fresh AML blasts to daunorubicin *in vitro* and was positively correlated with increased intracellular level of daunorubicinol,

a major catabolite of daunorubicin.[7] *CBR1* has also been suspected to be involved in the occurrence of anthracycline-related toxicities as non-synonymous SNP were associated with reduced metabolism of doxorubicin and daunorubicin, and as these metabolisms were correlated to the expression of carbonyl reductases.[8,9] In addition, polymorphisms in *CBR1* were correlated with altered pharmacokinetics with increased exposure to doxorubicin.[10] Finally, the cardioprotectant flavonoid 7-monohydroxyethyl rutoside was shown to behave as a CBR1 inhibitor.[11] Our data support the role of *CBR1* variants as predictors of anthracycline-related toxicity. The two SNP in CBR1 are situated very close to each other on chromosome 21 and are in high linkage disequilibrium, explaining the similar results obtained for the two variants.[7]

An important question when considering correlations of SNP with drug-induced toxicity is whether the polymorphisms actually impact on the expression level or functionality of the corresponding proteins. The effect of SNP on *ABCB1* has been reviewed by Leschziner *et al.*[12] Most studies concluded that there is no effect of rs2229109 (C1199A) on mRNA or protein expression. Concerning variants in *CBR1*, rare variants for both rs20572 and rs9024 were shown to be associated with lower mRNA expression than the most frequent alleles.[13] The rare variant of rs9024 was also shown to be associated with resistance to regulation by hsa-miR-291 but not by has-miR-574-5p.[14]

The strengths of our studies include the number of patients, the prospective collection of toxicity data in a randomized setting, and the fact that the SNPs analyzed have already been reported to be associated with toxicity, thus making some of these results a validation of previous hypothesis-generating studies. Limitations include the low number of patients in some groups and the number of statistical analyses performed without any correction for multiple tests. Differences in the toxicity parameters, which appear to be significantly correlated in the different analyzed groups (R-CHOP, R-ACVBP or all patients), are most probably due to the differences in the numbers of patients and their associated statistical power.

This study indicates the role for *ABCB1* and *CBR1* polymorphisms in the occurrence of severe myeloid and digestive toxicity in patients receiving CHOP-like regimens for the treatment of NHL. While confirmation of our results in other patient cohorts is required, the study design, with, in particular, the literature-based SNP selection, gives a partial validating value to our results already. Our study also confirms that the (R)-ACVBP regimen induces more toxicity in lymphoma patients than R-CHOP,[15] suggesting that markers predictive for toxicity would be of particular interest in patients receiving high-dose R-CHOP type regimens or in patients with pre-existing comorbidities.

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