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Candidate germline biomarkers of lenalidomide efficacy in mantle cell lymphoma: the FIL MCL0208 trial

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Abstract:
In the FIL MCL0208 phase III trial, lenalidomide maintenance (LEN) after transplantation (ASCT) in mantle cell lymphoma (MCL) improved progression-free survival (PFS) vs observation (OBS). The host pharmacogenetic background was analyzed to decipher whether single nucleotide polymorphisms (SNPs) of genes encoding transmembrane transporters, metabolic enzymes, or cell surface receptors might predict drug efficacy. Genotypes were obtained by real-time polymerase chain reaction (RT-PCR) in peripheral blood (PB) germ line DNA. Polymorphisms of either *ABCB1* or *VEGF* were found in 69% and 79% of 278 patients and predicted favorable PFS vs homozygous wild type (WT) in the LEN arm: 3-year PFS 85% vs 70% ($p < 0.05$) and 85% vs 60% ($p < 0.01$), respectively. Patients carrying both *ABCB1* and *VEGF* WT had the poorest 3-year PFS (46%) and overall survival (OS, 76%): in fact, in these patients LEN did not improve PFS vs OBS (3-year PFS 44% vs 60%, $p = 0.62$). Moreover, *CRBN* polymorphism (n = 28) was associated with lenalidomide dose reduction or discontinuation. Finally, *ABCB1*, *NCF4*, and *GSTP1* polymorphisms predicted lower hematological toxicity during induction, while *ABCB1* and *CRBN* polymorphisms predicted lower risk of grade {greater than or equal to}3 infections. This study demonstrates that specific SNPs represent candidate predictive biomarkers of immunochemotherapy toxicity and LEN efficacy after ASCT in MCL. This trial is registered at eudract.ema.europa.eu as 2009-012807-25.

Conflict of interest: COI declared - see note

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Candidate germline biomarkers of lenalidomide efficacy in mantle cell lymphoma: the Fondazione Italiana Linfomi MCL0208 trial

Running Title: Pharmacogenomics of lenalidomide in MCL

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KEY POINTS

- Polymorphic alleles of either ABCB1 or VEGF genes predicted better outcome in MCL patients receiving LEN after ASCT
- ABCB1, NCF4, GSTP1, and CRBN polymorphisms were associated to toxicity during induction and with LEN dose reductions

ABSTRACT

In the FIL MCL0208 phase III trial, lenalidomide maintenance (LEN) after transplantation (ASCT) in mantle cell lymphoma (MCL) improved progression-free survival (PFS) vs observation (OBS). The host pharmacogenetic background was analyzed to decipher whether single nucleotide polymorphisms (SNPs) of genes encoding transmembrane transporters, metabolic enzymes, or cell surface receptors might predict drug efficacy. Genotypes were obtained by real-time polymerase chain reaction (RT-PCR) in peripheral blood (PB) germ line DNA. Polymorphisms of either *ABCB1* or *VEGF* were found in 69% and 79% of 278 patients and predicted favorable PFS vs homozygous wild type (WT) in the LEN arm: 3-year PFS 85% vs 70% ($p < 0.05$) and 85% vs 60% ($p < 0.01$), respectively.

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Patients carrying both *ABCB1* and *VEGF* WT had the poorest 3-year PFS (46%) and overall survival (OS, 76%): in fact, in these patients LEN did not improve PFS vs OBS (3-year PFS 44% vs 60%, p = 0.62). Moreover, *CRBN* polymorphism (n = 28) was associated with lenalidomide dose reduction or discontinuation. Finally, *ABCB1*, *NCF4*, and *GSTP1* polymorphisms predicted lower hematological toxicity during induction, while *ABCB1* and *CRBN* polymorphisms predicted lower risk of grade ≥3 infections. This study demonstrates that specific SNPs represent candidate predictive biomarkers of immunochemotherapy toxicity and LEN efficacy after ASCT in MCL.

INTRODUCTION

Mantle cell lymphoma (MCL) is an aggressive, mature B-cell non-Hodgkin lymphoma (NHL) historically known for its poor long-term outcome.[1] In recent years, the introduction of novel treatment platforms has led to substantial improvement in patients' prognosis, almost uniformly including a maintenance phase which is now considered a critical stem of successful treatment.[2-5] Among maintenance regimens, lenalidomide, an oral immunomodulatory drug, has been demonstrated to have significant activity and a manageable safety profile, both alone or in combination with rituximab.[3-7] However, treatment response appears nonuniform across patients, and predicting the clinical drug profile remains an unmet need.

Many factors may impact inter-individual responsiveness to drugs, and the role of the host genetic background has also been investigated in lymphoma.[8-11] In particular, gene polymorphisms consist of a variation in the gene DNA sequence occurring in a population with a frequency ≥1% and resulting in changes in the expression, structure, and activity of the proteins encoded by these genes. Interestingly, previous reports have suggested that single nucleotide polymorphisms (SNPs) involving drug metabolic pathways are predictive of both response and toxicity in different histotypes of NHL.[8-15] This has been proved for

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several agents including anthracyclines,[12] monoclonal antibodies,[13,14] and immunomodulatory drugs including lenalidomide, which is impacted by SNPs in *ABCB1*, *ERCC5*, *XPA*, *GSTP1*, as demonstrated in multiple myeloma.[14,15]

The Fondazione Italiana Linfomi (FIL) MCL0208, a prospective multicenter, randomized phase III trial showed a significant progression-free survival (PFS) benefit of lenalidomide maintenance (LEN) vs observation (OBS) in young (<66 years old) previously untreated MCL patients.[3] The study enrolled 300 patients, and a large number of biological samples were centrally stored at fixed time points for the purpose of documenting minimal residual disease (MRD).[16] Moreover, several biological substudies were planned,[17,18] including a pharmacogenetic analysis. In particular, we investigated specific germ line polymorphisms of transmembrane transporters, metabolic enzymes, and cell surface receptors (*ABCB1*, *ABCG2*, *VEGFA*, *FCGR2A*, *NCF4*, *GSTP1*, *CRBN*) that might predict the efficacy and safety of immunochemotherapy and lenalidomide maintenance.

METHODS

Patients and treatment

This study was performed on biological samples collected from the phase III MCL0208 study (NCT02354313) sponsored by FIL. The trial enrolled 300 previously untreated MCL patients, aged 18–65 years, without clinically significant comorbidities. The planned treatment is shown in Figure S1. Briefly, patients received 3 R-CHOP-21, followed by R-high-dose cyclophosphamide (R-HD-CTX, 4 g/m^2), 2 cycles of R-high-dose Ara-C (2 g/m^2 q12h × 3d) and ASCT conditioned by using the BEAM or FEAM regimen.[3] After ASCT, responding patients (either complete [CR] or partial response [PR]) were randomly assigned to OBS or LEN, 15 mg days 1–21 every 28 days) for 24 months. Clinical results

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of the trial have already been published.[3] All patients provided written informed consent for the use of their biological samples for research purposes, in accordance with the Institutional Review Board's requirements and the Helsinki Declaration. The clinical trial, as well as the substudy, were approved by the ethics committees of all the enrolling centers. All the samples were centralized for scientific analysis in the hematological laboratory of Torino University.

Biological samples

Bone marrow (BM) and peripheral blood (PB) samples were collected at diagnosis and during follow-up, according to pre-planned time points of MRD analysis[16] (Figure S1). Biological samples were identified with a subject study number that can be traced or linked back to the subject only by the site investigator.

Minimal residual disease and mutational analysis

MRD monitoring was assessed on BM and PB samples by use of an ASO Real-Time quantitative PCR (RQ-PCR) approach (either on IGH or BCL-1/IGH rearrangements) at the indicated time points and evaluated according to the criteria of the EuroMRD standardization group.[16]

TP53 disruptions as well as *KMT2D* mutations were identified by NGS targeted resequencing and copy-number alteration analysis on CD-19–selected tumoral cells from BM baseline samples, or as previously described.[17]

Pharmacogenetic analyses

For the purposes of the pharmacogenetic study, germinal DNA was extracted by use of commercially available kits (QIAamp DNA Blood Mini Kit, Qiagen; Maxwell RSC Blood,

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Promega; DNAzol, Invitrogen). gDNA was extracted from different specimens, preferably selecting samples devoid of lymphoma infiltration. 193 patients were studied on a FU PB sample, mainly MRD negative or with very low levels of MRD (Table S5). When FU samples were not available, baseline PB was used (n=60), characterized by a median tumor infiltration of 3% by flow cytometry (range 0.03%-88%, table S5)

Specific germ line polymorphisms of transmembrane transporters, metabolic enzymes, and cell surface receptors were evaluated. The selected polymorphisms of the *ABCB1* gene were the following: rs1128503, rs2032582, and rs1045642. Additional SNPs belonging to other genes were chosen based on the mechanistic role of their encoded proteins in the pharmacokinetics and pharmacodynamics of lenalidomide. They included *ABCB2* rs2231142, *VEGF-A* rs699947, *FCGR2A* rs1801274, *NCF4* rs1883112, *GSTP1* rs1695, and *CRBN* rs1714327 and rs1705814 (Figure 1). The full list of investigated SNPs with their respective putative functions is shown in Table S2. For all of the SNPs, a minor allele frequency (MAF) of ≥0.3 in the European population was considered as a general criterion for selection.

Patients' genotypes with respect to the investigated SNPs were obtained by using specific TaqMan SNP Genotyping Assays (ABI, Applied Biosystems, Foster City, CA, USA) on an ABI Prism HT7900 Sequence Detection System instrument according to the manufacturer's instructions. Approximately 10% of samples were analyzed in duplicate and the results showed a concordance rate of >99%. The SDS Software (Applied Biosystems) was used to impute patients' genotypes, and allele frequencies and genotypes were calculated. The Hardy-Weinberg equilibrium (HWE) was checked by use of Pearson's χ^2 test (threshold value, p = 3.841) for each locus, while haplotypes and their frequencies were imputed with Arlequin software.[19] Finally, differences in genotype (or haplotype) distributions between groups of the study (i.e., the lenalidomide vs the observation arm) were evaluated by Pearson's χ^2 test.

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Toxicity evaluations and lenalidomide dose intensity

Toxic events were recorded according to the Common Terminology Criteria for Adverse Events, Version 4.0 (CTCAE v4.0). Only hematological toxicities were available in detail and were then correlated with pharmacogenetic data. In particular, granulocytes, platelets, and hemoglobin reductions were considered together across different treatment phases (induction, consolidation, and lenalidomide maintenance/observation). Infective toxicity was evaluated as a cumulative event according to the CTCAE v4.0. Lenalidomide dose intensity was calculated as the ratio between the effective received dose and the planned dose.

Statistical analysis

Due to the high number of collected biological and clinical variables, the entire dataset underwent systematic post-hoc quality control through data warehousing.[20]

Statistical analysis was carried out using R v4.0.0. For continuous variables, the Kruskal-Wallis test was used to compare medians between groups; for categorical variables, depending on the number, the chi-squared test or Fisher's exact test was used.

For survival analysis. progression-free survival (PFS) and overall survival (OS) were employed as clinical end points. PFS was calculated from the date of enrolment in the clinical study to the date of disease progression (event), death from any causes (event), or last follow up (censoring). OS was measured from the date of enrolment in the clinical study to the date of death from any cause (event) or last follow-up (censoring).[21] Survival was estimated with the Kaplan-Meier method and the log-rank test was applied to compare the survival distributions of the patients. The Cox proportional-hazards model was implemented for the univariate and multivariate survival analysis. For all statistical analyses, the level of significance was set at $p \leq 0.05$. The outcome data for the present analysis were updated as of December, 2017, as planned in the clinical trial.

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Data Sharing Statement

For original data, please contact simone.ferrero@unito.it

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RESULTS

Feasibility of the study

Overall, 300 patients were enrolled in the FIL-MCL0208 clinical trial: 93% of these patients (278/300) were included in the pharmacogenetic study due to the availability of adequate biological samples. In particular, 96% (197/205) of the randomized population, 97% (101/104) of patients randomized to LEN, and 95% (96/101) of those randomized to OBS were genotyped (Figure S2). The main clinical features of these patients are described in Table S1.

Pharmacogenetic analyses

Allele and genotype frequencies of all the investigated SNPs are detailed in Table 1. The MAF values did agree with those already calculated in European populations, without significant differences between the LEN and OBS arms. Of note, all SNPs were in HWE, except for the *CRBN* locus rs1705814.

The expected linkage between the three *ABCB1* SNPs was confirmed (Tables S3), with percentage values in the LEN/OBS arms of 18.8%/16.7%, 51.5%/50.0%, and 29.7%/33.3% for wild type homozygous (i.e., CGC/CGC), heterozygous polymorphic (i.e., CGC/TTT), and polymorphic homozygous (i.e., TTT/CTT) patients, respectively. No significant differences in haplotype distribution were observed between the enrolled and randomized population, as well as between the two arms ($\chi^2 = 0.264$, $p = 0.876$).

Impact of SNPs on treatment efficacy

Among the investigated polymorphisms, only two, namely *ABCB1* rs2032582 and *VEGF* rs699947, were associated with lenalidomide efficacy, in terms of both PFS and OS. In the randomized population, 60 (31%), 107 (54%), and 30 (15%) patients were wild-type homozygotes (HoWT), heterozygotes (HePOL), and polymorphic homozygotes (HoPOL) for the *ABCB1* locus. For the *VEGF* rs699947 locus, 42 patients (21%), 96 (49%), and 59 (30%) were HoWT, HePOL, and HoPOL, respectively (Table S4).

Survival analysis was first performed by separately comparing these three groups (FigureS4), but HoPOL and HePOL patients were then grouped together, as these patients showed superimposable outcomes. In the randomized population, 137 patients (70%) carried at least one polymorphic allele for the *ABCB1* rs2032582 locus. Interestingly, HoPOL (WW) and HePOL (GW) had better outcomes when compared with HoWT (GG) in the LEN arm (3-year PFS 85% vs 70%, p = 0.047; and 3-year OS 98% vs 90%, p = 0.026) but not in the OBS arm (see Figure 2 A–D).

Similarly, 155 patients (79%) carried at least one variant allele of the *VEGF-A* locus, so that HoPOL (CC) and HePOL (AC) had better outcomes in the LEN arm vs HoWT (3-year PFS 85% vs 60%, p = 0.0021; and 3-year OS 90% vs 86,5%, p = 0.094) but not in the OBS arm (Figure 2 E–H).

Accordingly, patients carrying variant genotypes of either gene (*ABCB1* or *VEGF-A*) showed a trend towards deeper MRD clearance by RQ-PCR in BM after 6 months of LEN, when compared with HoWT (Figure S3). In this population, as well as in the overall series, HoWT did not differ from HoPOL and HePOL based on baseline clinical features, classical prognosticators (including *TP53* aberrations), *ABCB1*, and *VEGF-A* polymorphisms (Table S3).

Moreover, by analyzing the combinatorial effect of each polymorphism in the same patients,

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only the small group of “double WT” cases (i.e., *ABCB1* HoWT and *VEGF-A* HoWT) receiving lenalidomide were actually associated with poor survival, in terms of both PFS and OS (Figure 3). Therefore, we reconsidered the efficacy of lenalidomide maintenance by stratifying the randomized population based on its pharmacogenomic background. Survival analysisshowed that patients carrying at least one variant allele of *ABCB1/VEGFA* polymorphic loci experienced the highest benefit from LEN (PFS HR = 0.41 [95% CI 0.22–0.75], p = 0.004, vs PFS HR = 0.51 [95% CI 0.30–0.87], p = 0.012) of the entire randomized population.[28] On the other hand, from the limited number of “double WT” patients (n = 17), this genotype seemed to confer no benefit at all in the LEN vs the OBS arm (p = 0.632), as shown in Figure 4.

Impact of SNPs on treatment toxicity

Lenalidomide dose reduction

Dose reduction of lenalidomide was significantly associated only with the *CRBN rs1705814* genotype. In the studied population, 113 patients (41%) were HoWT (TT), 91 (33%) HePOL (TC), and 73 (26%) HoPOL (CC) (Table S4). It is worth noting that 28 patients randomized to LEN (28%) were HoPOL for the *CRBN* rs1705814 locus and had a higher risk of major lenalidomide dose reduction (more than 66%) or discontinuation during the maintenance phase with respect to HoWT/HePOL (OR 3.24, IC 1.69–6.21, p = 0.013). Nonetheless, in the LEN randomization arm, no statistically significant impact of the *CRBN* rs1705814 SNP was observed on either hematological toxicities or infections (see Table 2).

Hematological toxicity

Three polymorphisms were associated with hematological toxicity in the whole population of patients: *ABCB1* rs2032582*, NCF4* rs1883112, and *GSTP1* rs1695 (see Table S3 for

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the distribution of genotypes). Indeed, 46 patients (17%) carrying *ABCB1* TT/AT/AA genotypes had a lower risk of hematological toxicity after the first R-CHOP cycle than did patients carrying HePOL/HoWT (OR for G ≥3 toxicity 0.39, IC 0.15–0.88, p = 0.033). Moreover, 162 patients (58%) carried *NCF4* AG/GG genotypes and were exposed to an overall lower risk of hematological toxicities during induction (within the R-HD-CTX cycle) when compared with HoWT (OR 0.56, IC 0.34–0.92, p = 0.024) (see Table 2). Similarly, 28 patients (10%) carried the *GSTP1* GG HoPOL genotype and were exposed to a lower risk of toxicities than were HePOL/HoWT (OR 0.35, IC 0.15–0.79, p = 0.014 (Table 2). Interestingly, by combining the postulated protective effect of both SNPs, we were able to identify a subgroup of patients (*NCF4* and *GSTP1* HoWT, n = 48) at higher risk of hematological toxicity during induction (OR 2.26, IC 1.09–4.83, p = 0.031). No impact of these SNPs was observed during later treatment phases (R-HD- ARAC), nor on hematological recovery after ASCT.

Infective toxicity

Two polymorphisms, *ABCB1* rs1045642 and *CRBN rs1705814*, were associated with infections in the whole series of patients during chemo-immunotherapy. In the studied population, the following allele frequencies were found: for *ABCB1 c.3435*.C>T, 70 patients (25%) were HoWT (CC), 143 (51%) HePOL (CT), and 65 (23%) HoPOL (TT). For *CRBN* rs1705814 T>C, 113 patients (41%) were HoWT (TT), 91 (33%) HePOL (TC), and 73 (26%) HoPOL (CC) (Table S3). The 65 out of 278 patients carrying the *ABCB1 TT* genotype (Table S4) were exposed to a lower risk of infections than were CT/CC individuals (OR 0.53, IC 0.30–0.95, p = 0.030). Similarly, 73 patients HoPOL for the *CRBN* rs1705814 locus had a lower risk of severe infections than did those who were HoWT/HePOL (OR for G≥3 toxicity 0.39, IC 0.22–0.68, p = 0.001). On the other hand, none of the SNPs predicted the onset of infections during lenalidomide maintenance.

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DISCUSSION

The pharmacogenetic study of the FIL MCL0208 phase III randomized trial showed that SNPs of genes encoding specific cellular proteins may be associated with clinical outcome in younger MCL patients receiving lenalidomide maintenance after ASCT. Most importantly, we observed that

- *ABCB1 c.2677* SNP (transmembrane transporter) was significantly associated with better PFS and OS;
- *VEGF-A c.2055* SNP (angiogenic factor) was significantly associated with better PFS;
- by combining *ABCB1* and *VEGF-A* genotypes, a small subgroup of MCL patients can be identified who did not benefit from lenalidomide maintenance;
- moreover, *CRBN rs1705814* SNP (a lenalidomide molecular target) was associated with a higher risk of lenalidomide dose reduction or discontinuation.

Notably, no significant differences in genotype distribution were observed between the two randomization arms, resetting any possible bias due to unbalanced stratification of patients and reducing the weight of confounding factors in the lenalidomide maintenance phase. In addition, some SNPs (namely *ABCB1 c.3435C>T*, *NCF4 c.368G>A*, *CRBN* rs1705814 *T>C* and *GSTP1 c.313.A>G*) were associated with hematological and infective toxicity during the immunochemotherapy phase preceding the randomization.

In recent years, several studies have underlined the potential role of interindividual genetic differences in shaping treatment response and toxicity in lymphoma. [22,23] Nonetheless, to

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date little pharmacogenetic knowledge is available in the MCL literature,[24-27] and no data relative to lenalidomide treatment in MCL have been published. On the other hand, several pharmacogenetic studies have been published, mainly in multiple myeloma, assessing the relation of lenalidomide activity with polymorphisms of *CRBN*, one of lenalidomide's most investigated molecular targets.[28-31] Thus, this study provides the first pharmacogenetic data related to lenalidomide treatment in MCL by investigating the effects of candidate genes and polymorphisms on both efficacy and toxicity. Because some of the genes may play a role in immunochemotherapy outcomes and tolerability, the investigated panel of loci was also assessed for these effects in the whole population of patients.

Our main findings suggested a key role of *ABCB1* and *VEGF-A* polymorphisms in enhancing the clinical activity of lenalidomide maintenance after ASCT in MCL. Biologically, we might hypothesize that variant alleles of *ABCB1*, a transmembrane drug transporter, leading either to decreased gene expression in gut cells and renal tubules or to reduced ejection activity of the transporter,[32-34] resulted in greater lenalidomide bioavailability, leading to an increased pharmacological effect due to higher plasma and tissue drug concentrations. This hypothesis is in line with published literature of lenalidomide pharmacokinetics in MM[35,36], but in this study no gut or renal tissue was available to investigate the hypothesis.

Similarly, the polymorphic genotype of *VEGF-A* might lead to decreased gene expression (as suggested in other scenarios [37-39]), resulting in decreased stimulation by this key cytokine in the PI3K-Akt pathway.[40] Unfortunately, adequate lymphoma tissue for gene expression analysis was available only for a minority of the patients of this trial and so no statistically relevant conclusions might be drawn from these data[41]. Anyway, given the direct inhibitory effect of lenalidomide on VEGF-A–mediated Akt phosphorylation, the presence of the *VEGF-A C* polymorphic allele might additively enhance lenalidomide's pharmacological effect. Therefore, we hypothesized that the absence of both polymorphic genotypes might lead to lower lenalidomide and higher VEGF-A concentrations, and thus

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act as a predictive biomarker of poor response to lenalidomide in MCL. Accordingly, patients carrying neither *ABCB1* nor *VEGF-A* polymorphisms (actually the 9% of the series) did not benefit from maintenance therapy with lenalidomide when compared with observation only (Figure 4B). Thus, these results suggest a possible means of selecting patient candidates for lenalidomide, to improve its efficacy and concomitantly reduce the risk of toxicity. More cumbersome is the association found between the *CRBN* rs1705814 polymorphic genotype and lenalidomide dose reduction. We might hypothesize that this genotype could reduce the intracellular expression of cereblon, the molecular target of lenalidomide, and so also the minimum lenalidomide dose required to obtain the therapeutic effect. Anyway, our analysis was not able to show any statistically significant association between this SNP and higher risk of hematological or infective toxicities, possibly explaining the observed lenalidomide dose reduction. Further analyses are required to explain this finding, including the roles of different toxic events not reported in the trial eCRFs, as well as a wider validation on an external, well annotated patients population, such as for example the recently completed EuMCLNet “R2 Elderly” trial[42]. Finally, we cannot exclude the possibility that other polymorphisms might serve as possible biomarkers of lenalidomide efficacy, but none of the analyzed genes other than *ABCB1* and *VEGF-A* showed promising survival trends that deserved to be further investigated (data not shown).

Considering the standard chemotherapeutic regimens administered to patients before ASCT, it was hypothesized that some of the SNPs identified and evaluated in the present work could also act as predictive markers of tolerability, because proteins encoded by the corresponding genes are involved in the transmembrane transport (ABCB1) or detoxification (GSTP1 and NCF) of cyclophosphamide, vincristine, and doxorubicin. Indeed, we found that at least three loci in different genes were significantly associated with hematological toxicities during the induction phase, with an increased risk with patients

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carrying WT genotypes. That risk was coupled with an increased incidence of severe infections, at least when considering *ABCB1*.

Our study has some strengths. First, the robustness and reproducibility of real-time PCR analysis ensures its theoretical large-scale applicability in clinical practice across different labs, as the present analyses can be carried out on several instrumental platforms,including the most recent ones. Easy standardization, a patient-friendly PB source, and overall limited costs also favor this approach. Furthermore, it is intriguing to derive predictive biomarkers by investigating the genetic background of every single patient, in addition to the intrinsic alterations of tumor cells, and hence allow wider approaches to obtain somatic DNA (i.e., buccal swab). Nevertheless, although promising, our strategy identified only a limited number of cases with an adverse genotype, and we totally lack functional studies; therefore, an external validation on an independent series of patients receiving lenalidomide is needed for validation of these candidate biomarkers. Moreover, we acknowledge to have investigated in this cohort only a finite number of gene polymorphisms, selected on the basis of data already available in the literature, and probably not representing all the pharmacogenomics targets potentially involved in lenalidomide's mechanism of action. In this regard, current genome-wide sequencing tools may be applied in the future as more convenient technical approaches to study, in one run, larger panels of different gene polymorphisms. Finally, the number of MCL patients enrolled in this study is the largest so far. However, the sample size of this study may have limited the comprehensive detection of associations with less common alleles. Additional studies on larger MCL populations will help confirm the current findings and may reveal additional variants that influence drug-associated efficacy and toxicity. Overall, pharmacogenomics might play a role in MCL therapy, enabling clinicians to provide tailored treatment based on individual patients' genetic profiles, possibly complementing classical mutational and expression analysis carried out on tumor cells.[17,43] This paradigm might also be scalable to

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several novel drugs currently employed in MCL treatment (such as Bruton's tyrosine kinase inhibitors or bispecific antibodies),[44,45] as well as in other lymphomas[46] or in multiple myeloma, where lenalidomide is widely used.[47,48] In fact, the comprehensive identification of patients who benefit the most from lenalidomide or other therapeutic approaches would not only bring clinical benefits and improved quality of life to our patients but would also be valuable from a pharmacoeconomic perspective.

In conclusion, this study demonstrates that pharmacogenetic analysis of PB samples could easily yield predictive biomarkers of poor response to lenalidomide maintenance after ASCT in MCL patients. Despite some methodological limitations, this approach is shown to be promising and theoretically scalable to different clinical contexts. Thus, it is a perfect example of how precision medicine might present an exceptional opportunity for our patients in the near future.

AUTHORSHIP CONTRIBUTIONS

GP, SG, SC, ADP, and ML conceived and designed the study; SF, DG, ADR, AR, VS, FC, CB, MB, VZ, MGdS, LA, PT, FB, AF, BP, SC, and ML enrolled patients and provided biological samples. EA, BA, and EG performed experiments; SF, DG, MP, GMZ, MG, and ADP collected and analyzed data; GDL performed statistical analysis; and SF, DG, MP, RT, GP, SG, ADP, and ML wrote the paper. All the authors approved the final version of the manuscript.

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CONFLICT OF INTEREST DISCLOSURES

SF: Janssen (consultancy, advisory board, speaker's honoraria, research funding); EUSA Pharma (consultancy, advisory board, speaker's honoraria); Gilead, Morphosys (research funding); Incyte, Clinigen (advisory board); Servier, Gentili (speaker's honoraria).

GAP: Speaker's fees from AbbVie, Bristol Myers Squibb (BMS), Incyte, and Novartis; service on advisory boards for Abbvie Orphan Pharmaceuticals, AstraZeneca, BMS, and Novartis; support for attending meetings from Abbvie, BMS, Janssen, and Novartis.

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LA: Consulting or advisory role: Roche, Janssen-Cilag, Verastem, Incyte, EUSA Pharma, Celgene/Bristol Myers Squibb, Kite/Gilead, and ADC Therapeutics; speakers' bureau: EUSA Pharma, Novartis; research funding: Gilead Sciences.

VRZ: Consulting or advisory role: Takeda, Janssen, MSD, Roche, Servier, and Kite; travel, accommodations, expenses: Takeda and Janssen.

SG: Speaker at events supported by Jazz, Janssen, Pfizer, Novartis, Incyte, Astra Zeneca, Abbvie.

FC: Advisory or consulting role for Roche, Astra Zeneca; speaker: Servier; travel accommodation:

Takeda, Astra Zeneca, Roche.

CB: Consulting or advisory role: Abbvie, Astra Zeneca; travel, accommodations, expenses: Janssen.

The other authors have no relevant conflicts of interest to disclose.

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Legends:

Figure 1. Depiction of the biological functions of the proteins encoded by the investigated genes and impact of relative polymorphisms.

ROS, reactive oxygen species; GSTP1, glutathione-S-transferase Pi1; ATP, adenosine triphosphate; FCGR, Fc Gamma Receptor; VEGF-A, vascular endothelial growth factor A, ABCB1, ATP binding cassette subfamily B member 1; Ub, ubiquitin; eNOS, endothelial nitric oxide synthase.

Figure 2. Association between *ABCB1* or *VEGF-A* grouped genotypes and survival in a randomized population. PFS stratified by *ABCB1* genotypes in the LEN (A) and OBS arms (C); OS stratified by *ABCB1* genotypes in the LEN (B) and OBS arms (D); PFS stratified by *VEGF-A* genotypes in the LEN (E) and OBS arms (G); OS stratified by *VEGF-A* genotypes in the LEN (F) and OBS arms (H).

PFS: Progression-free survival; OS: overall survival; LEN: lenalidomide; OBS: observation.

ABCB1: HoWT=GG, HePOL=GW, HoPOL=WW; ***VEGF-A***: HoWT=AA, HePOL=AC, HoPOL=CC

Figure 3. Association between combined *ABCB1/VEGF-A* genotypes and survival in the LEN population. PFS (A) and OS (B) stratified by the combination of *ABCB1* and *VEGF-A* genotypes.

PFS, progression-free survival; OS, overall survival; POL, polymorphic; HoWT, homozygous wild type.

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Figure 4. Progression-free survival by randomization arm stratified on the pharmacogenomic background. PFS stratified by *ABCB1* and *VEGF-A* genotypes in the LEN and OBS arms: *ABCB1*/*VEGF-A* HoWT (A) and *ABCB1*/*VEGF-A* POL (B).

PFS, mprogression-free survival; POL, polymorphic; HoWT, homozygous wild type; LEN, lenalidomide; OBS, observation; HR, hazard ratio.

Table 1. Allele and genotype frequencies of SNPs evaluated in the whole series.

SNPs, single-nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency.

Table 2. Association of investigated SNPs with toxic events.

SNPs, single-nucleotide polymorphisms; HoPOL, homozygote polymorphic; HePOL, heterozygote polymorphic; HoWT, homozygote wild-type; OR, odds ratio; IC, confidence interval.

		Whole population		Lenalidomide Arm		Observation Arm			
		MAF	**HWE**	**MAF**	**HWE**	**MAF**	**HWE**	***p****	***chi²****
ABCB1	c.1236	0.444	0.881	0.436	0.113	0.448	1.811	0.484	1.454
ABCB1	c.2677	0.428	1.464	0.426	0.283	0.422	2.923	0.680	0.771
ABCB1	c.3435	0.491	0.236	0.485	0.008	0.521	0.181	0.732	0.625
VEGF	c.2055	0.563	0.499	0.559	0.421	0.526	0.994	0.439	1.645
ABCG2	c.421	0.939	0.002	0.926	0.56	0.964	0.137	0.157	2.000
FCGR2A	c.497	0.404	0.459	0.411	0.641	0.389	1.082	0.400	1.834
NCF4	-368	0.359	0.108	0.401	0.532	0.316	0.525	0.266	2.652
GSTP1	313	0.327	0.237	0.317	1.730	0.300	0.048	0.660	0.831
CRBN	rs1714327	0.285	1.046	0.292	1.316	0.268	1.268	0.817	0.404
CRBN	rs1705814	0.428	29.977[#]	0.426	13.563[#]	0.379	5.456[#]	0.390	1.885

Table 1

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Polymorphism	*ABCB1 c.2677 G>T/A*	*NCF4 c.368G>A*	*GSTP1 c.313.A>G*	*CRBN rs1705814 T>C*
Hematological toxicity during the induction phase	**HoPOL – OR 0.39** (IC 0.15-0.88) p = **0.033**	**HoPOL/HePOL - OR 0.56** (IC 0.34-0.92) p = **0.024**	**HoPOL - OR 0.35** (IC 0.15-0.79) p = **0.014**	
Combined effect		**HoWT - OR 2.26** (IC 1.09-4.83) p = **0.031**		
Infective toxicity	**HoPOL – OR 0.53** (IC 0.30-0.95) p = **0.030**			**HoPOL – OR 0.39** (IC 0.22-0.68) p = **0.001**
Lenalidomide dose reduction				**HoPOL - OR 3.24** (IC 1.69-6.21) p = **0.013**

Table 2

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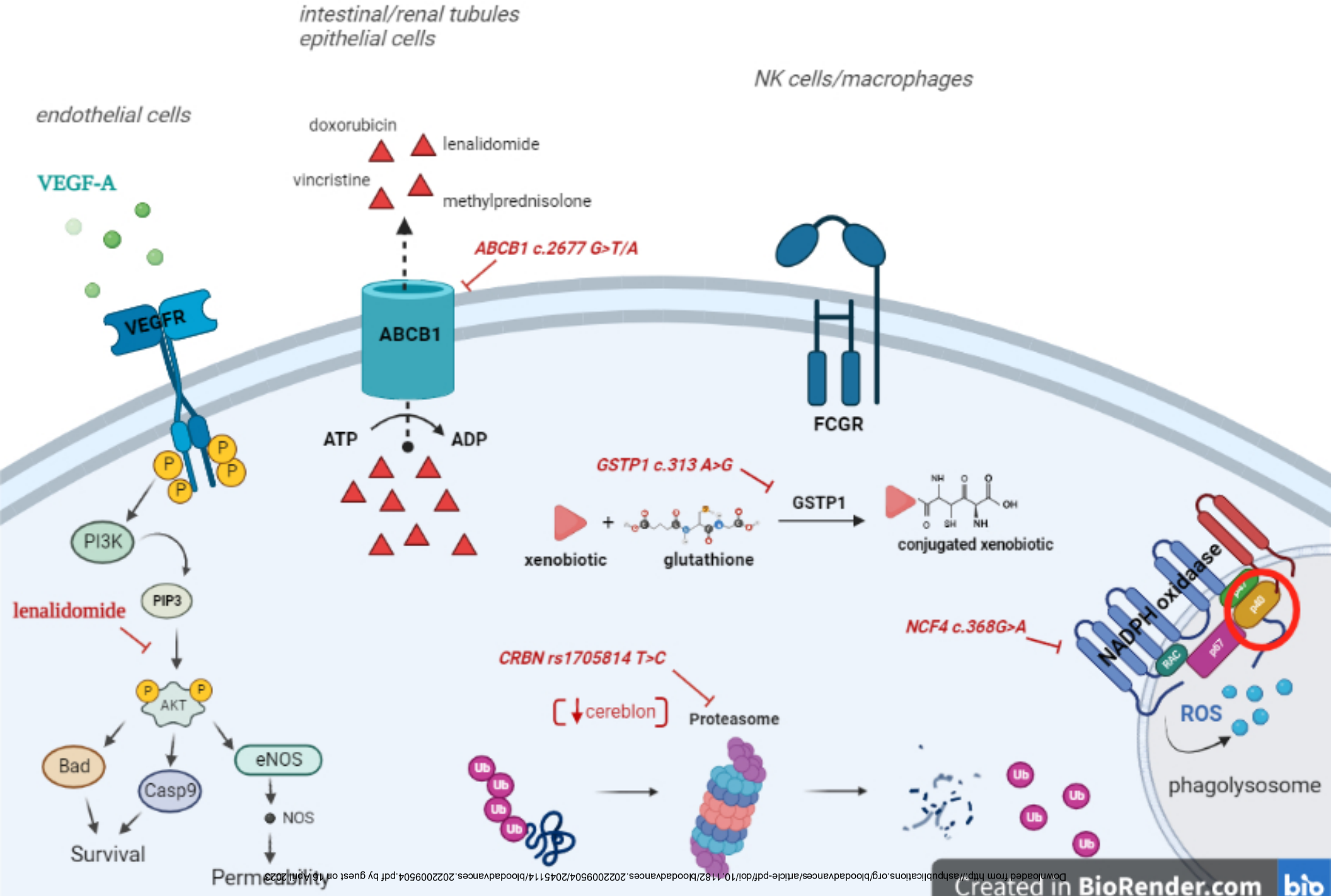
Figure 1



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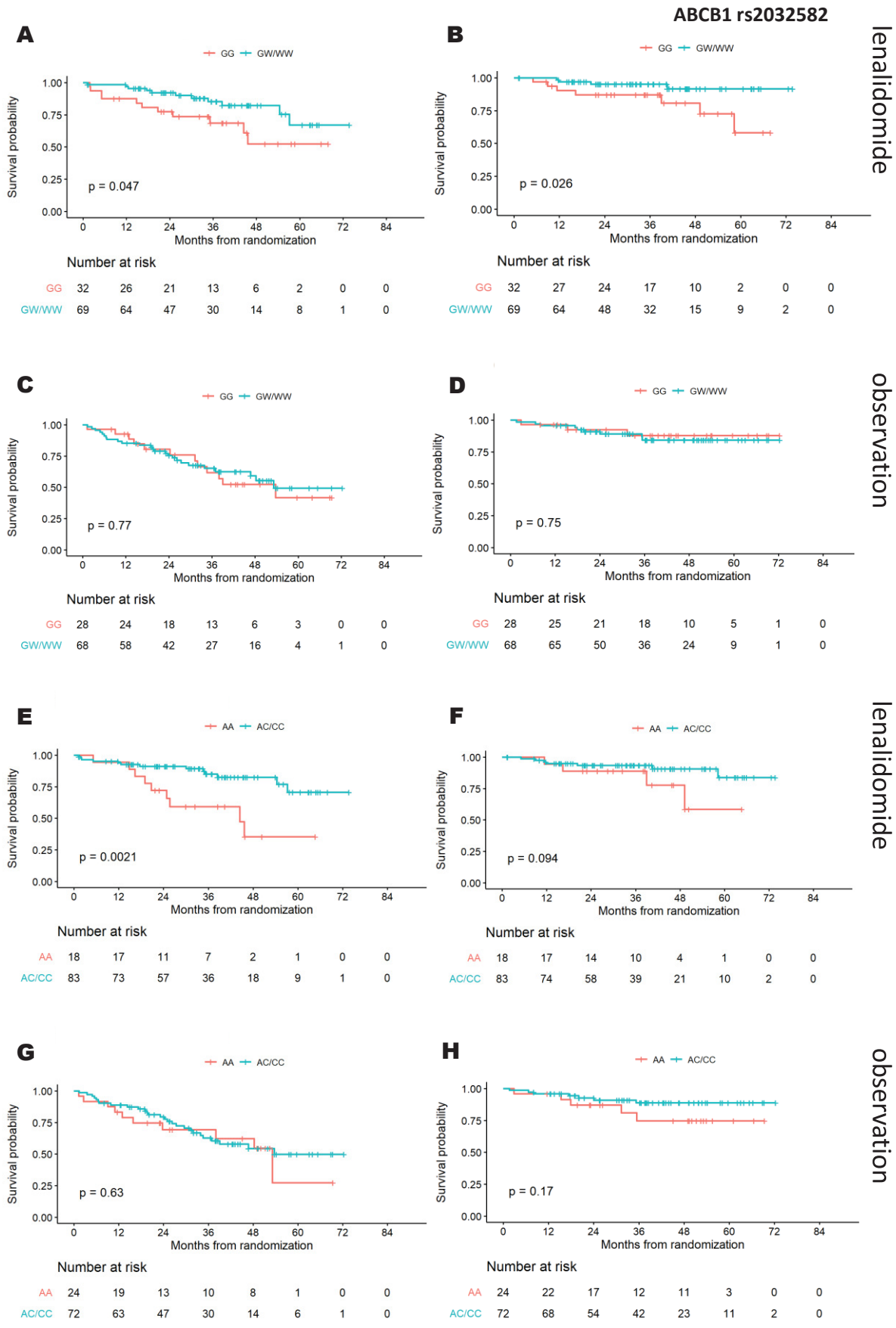

Figure 2

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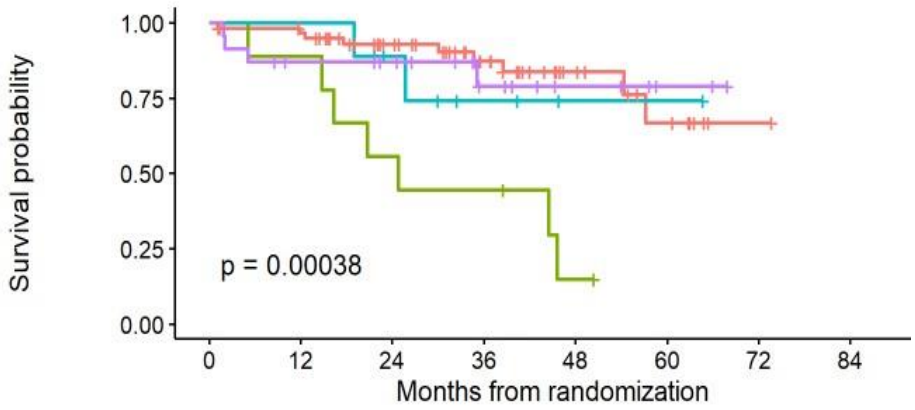
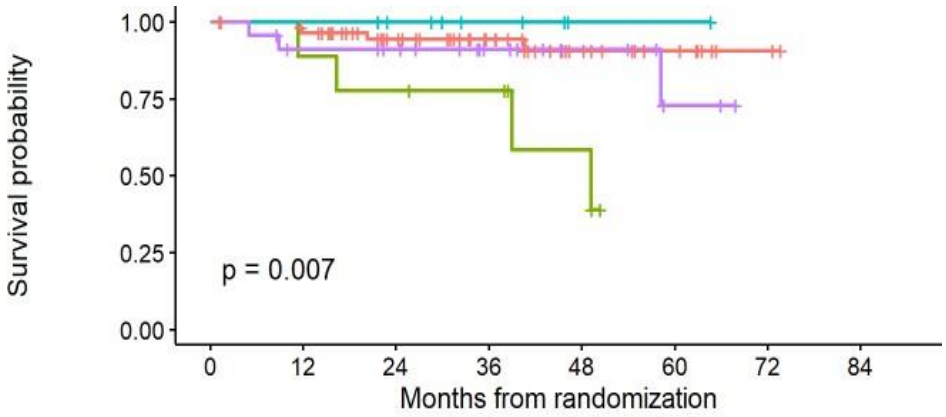
(A) PFS



Number at risk

ABCB1/VEGF-A POL	60	55	41	27	13	7	1	0
ABCB1/VEGF-A HoWT	9	8	5	4	1	0	0	0
ABCB1 POL/VEGF-A HoWT	9	9	6	3	1	1	0	0
ABCB1 HoWT/VEGF-POL	23	18	16	9	5	2	0	0

(B) OS



Number at risk

ABCB1/VEGF-A POL	60	55	41	28	14	8	2	0
ABCB1/VEGF-A HoWT	9	8	7	6	3	0	0	0
ABCB1 POL/VEGF-A HoWT	9	9	7	4	1	1	0	0
ABCB1 HoWT/VEGF-POL	23	19	17	11	7	2	0	0

Figure 3

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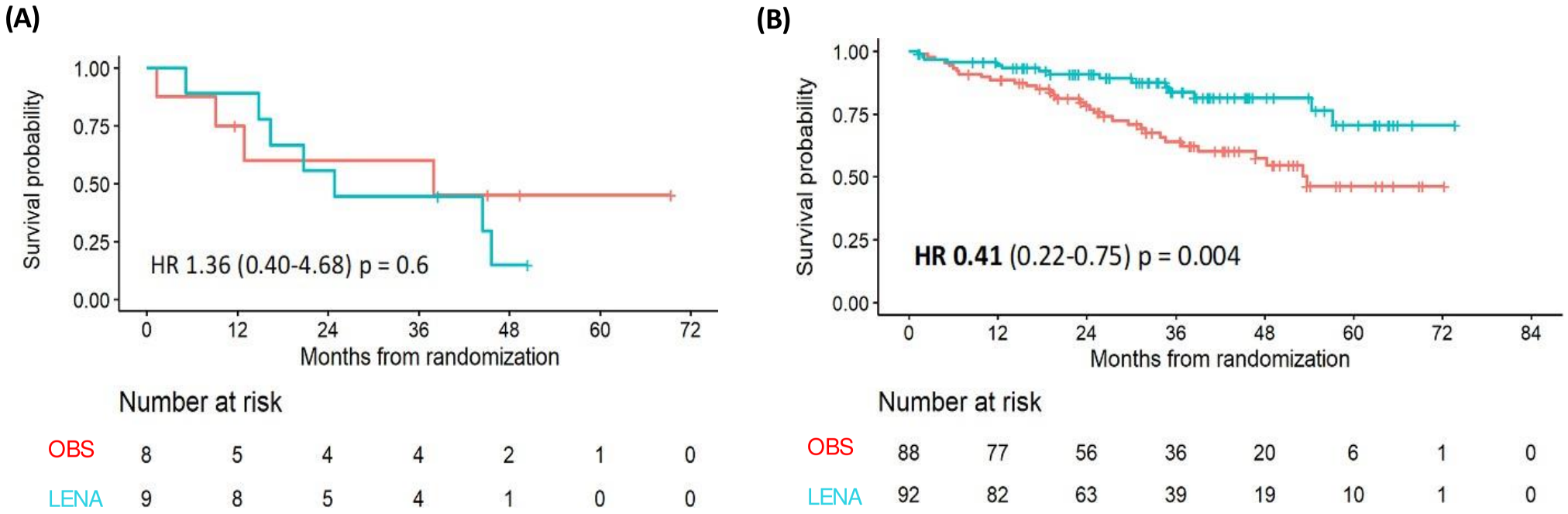

Figure 4

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