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Impact of *ABCB1* single nucleotide polymorphisms 1236C>T and 2677G>T on overall survival in *FLT3* wild-type *de novo* AML patients with normal karyotype

Running title: ABCB1 polymorphisms in AML

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Summary

Drug resistance is a clinically relevant problem in the treatment of acute myeloid leukemia (AML). We have previously reported a relationship between single nucleotide polymorphisms of *ABCB1*, encoding the multi-drug transporter P-glycoprotein, and overall survival (OS) in normal karyotype (NK)-AML. Here we extended this material, enabling subgroup analysis based on *FLT3* and *NPM1* status, to further elucidate the influence of *ABCB1* SNPs. 201 *de novo* NK-AML patients were analyzed for 1199G>A, 1236C>T, 2677G>T/A and 3435C>T, and correlations to outcome were investigated. *FLT3* wild-type 1236C/C patients have significantly shorter OS compared to patients carrying the variant allele; medians 20 vs. 49 months, respectively, $p=0.017$. There was also an inferior outcome in *FLT3* wild-type 2677G/G patients compared to patients carrying the variant allele, median OS 20 vs. 35 months, respectively, $p=0.039$. This was confirmed in Cox regression analysis. Our results indicate that *ABCB1* 1236C>T and 2677G>T may be used as prognostic markers to distinguish relative high risk patients in the intermediate risk *FLT3* wild-type group, which may contribute to future individualizing of treatment strategies.

Introduction

Acute myeloid leukemia (AML) is a hematologic malignancy characterized by failed differentiation and uncontrolled proliferation of hematopoietic myeloid progenitor cells. Although many patients reach initial complete response to treatment, more than two-thirds of AML patients suffer from relapse and 70-80% ultimately dies of their disease. The current standard treatment of AML consists of a combination of the nucleoside analog cytosine arabinoside (AraC) and an anthracycline such as Daunorubicin or Idarubicin, followed by additional chemotherapy and/or bone marrow transplantation. Due to the high relapse rate, but also to treatment related mortality, the 5-year overall survival rate in AML is only 40% and less than 15% in AML patients above the age of 65 (Juliussen, *et al* 2009).

Development of multidrug resistance to cancer chemotherapy is a clinically relevant problem when aiming for successful treatment of AML. As demonstrated by our recently

published study on genetic variation in genes encoding enzymes metabolizing AraC, SNPs affecting drug activation and/or inactivation may be important factors for drug sensitivity and the subsequent outcome in the treatment of AML (Falk, *et al* 2013). Also, excessive drug efflux from the cells due to increased expression of P-glycoprotein, encoded by the *ABCB1* gene, is a well-known mechanism of drug resistance. P-glycoprotein mediates extrusion of cytotoxic drugs of different structures and mechanisms of action, including vinca alkaloids and anthracyclines, and cross resistance occurs (Germann 1996). Several single nucleotide polymorphisms (SNPs) in the *ABCB1* gene have been identified and related to changes in P-glycoprotein expression and activity, including 1236C>T (silent, rs1128503), 2677G>T/A (Ala893Ser, rs2032582) and 3435C>T (silent, rs1045642) (Hoffmeyer, *et al* 2000, Kim, *et al* 2001, Tanabe, *et al* 2001). An alteration of transport activity due to SNPs might convey differences in cell sensitivity towards environmental toxins and susceptibility to cancer, but may also affect the sensitivity to anticancer agents and thereby subsequently have an impact on treatment outcome (Evans and McLeod 2003, Marzolini, *et al* 2004, Robert, *et al* 2005).

Based on the characterization of cytogenetic aberrations, AML patients are classified into three prognostic groups, low, high and intermediate risk, with the latter being the predominant one including patients with aberrations of unclear significance and patients with normal karyotype. Normal karyotype AML patients (NK-AML) constitute about 45% of all cases of *de novo* AML, and these intermediate risk patients are a heterogeneous group in which some patients reach and maintain complete remission, while others rapidly relapse (Bacher, *et al* 2006, Grimwade, *et al* 2010, Grimwade, *et al* 2001). During the last years, internal tandem duplications (ITDs) in fms-related tyrosine kinase 3 (*FLT3*), mutations in the nucleophosmin 1 (*NPM1*) and CCAAT/enhancer binding protein alpha (*CEBPA*) genes have been described as markers of clinical outcome in *de novo* NK-AML, with *FLT3*-ITD correlating to a poor outcome, and *NPM1* and/or *CEBPA* mutations in the absence of *FLT3*-ITD being associated to a more favorable prognosis (Bienz, *et al* 2005, Falini, *et al* 2005, Mrozek, *et al* 2007, Nakao, *et al* 1996, Renneville, *et al* 2009). However, there is a large group of NK-AML patients with ambiguous genetic status for these prognostic markers, and there is an

obvious need for further biomarkers to guide the clinician in individual treatment decisions for this heterogeneous intermediate risk patient group.

We have previously reported that the *ABCB1* single nucleotide polymorphisms 1236C>T and 2677G>T/A have an influence on overall survival in *de novo* NK-AML (Green, *et al* 2012). Several other studies have investigated the importance of genetic variation in *ABCB1* in AML treatment, with conflicting results (Hur, *et al* 2008, Illmer, *et al* 2002, van den Heuvel-Eibrink, *et al* 2001, van der Holt, *et al* 2006). We now aim to confirm our results by extending our material of *de novo* NK-AML patients, making subgroup analysis possible, to further clarify the potential of *ABCB1* SNPs as prognostic factors and tools for individualization of chemotherapy in the treatment of AML.

Materials and Methods

Patients

This study was based on a previously collected and published material of 100 patients with *de novo* NK-AML (Green, *et al* 2012), which was further increased by another 110 patients. After collection, patients treated with non-curative intent were excluded, leaving a total of 201 *de novo* NK-AML patients, retrospectively collected from four different Swedish centers and diagnosed between 1988 and 2009. Blood and bone marrow samples collected at diagnosis before treatment initiation was used for genotyping. Clonal aberration evaluation was carried out according to the International System for Human Cytogenetic Nomenclature (ISCN 2005), and *NPM1* and *FLT3* status were also determined. Patients diagnosed 2005 or later were treated according to national guidelines (<http://www.sfhem.se/Filarkiv/Nationella-riktlinjer>, accessed 2013-08-30). Thus, the large majority received induction treatment regimens including Daunorubicin 60mg/m²/day for three days combined with AraC as 1000mg/m² twice a day in 2h i.v infusions for 5 days. Before 2005, regional guidelines most commonly included AraC doses of 200 mg/m² as 24 h i.v infusions for 7 days, together with Daunorubicin or Idarubicin for three days (Wahlin, *et al* 2009). 101 (50.2%) of the patients were diagnosed before 2005, and 100 (49.8%) were

diagnosed 2005 or later. The response after chemotherapy was evaluated as non-complete remission (no CR) or complete remission (CR) according to the ELN definitions (Dohner, *et al* 2010). Accordingly, patients with treatment failure, regardless of cause, were defined as no CR. Information on response was missing in 1 patient. Survival times were calculated as the time from diagnosis until an event (progression or death) or the latest follow up date. Patients receiving allogeneic stem cell transplantation (allo-SCT) (n = 57) were censored at the time of transplantation in the survival analysis. The study was approved by the local ethical committee and conducted in compliance with the Helsinki declaration. Patient characteristics and treatment details are summarized in **Table I**.

ABCB1 analysis

DNA was isolated and shipped to Clinical Pharmacology at Linköping University, where all patients were genotyped. The *ABCB1* single nucleotide polymorphisms 1199G>A (Ser400Asn, *rs2229109*), 1236C>T (silent, *rs1128503*), 2677G>T/A (Ala893Ser, *rs2032582*), and 3435C>T (silent, *rs1045642*) were analyzed using Pyrosequencing as previously described (Green, *et al* 2006, Green, *et al* 2008). Briefly, HotStar Taq Master mixture (VWR International) was used for PCR amplification and all reactions were carried out on a Mastercycler gradient (Eppendorf) in a total volume of 25 µl. A final primer concentration of 0.4 µM, a final MgCl₂ concentration of 1.5 mM, and an annealing temperature of 58°C was used. The SNPs were analyzed using a Pyrosequencing PSQ96MA instrument (Qiagen, Uppsala, Sweden) according to the manufacturer's protocol and as previously described (Green, *et al* 2006, Green, *et al* 2008). In short, single stranded biotinylated PCR template was prepared and sequencing primer was annealed at 80°C for 2 min. Enzyme and substrate mixtures were added and sequencing was performed by adding dNTPs in a predefined dispensation order.

FLT3 and NPM1 genotyping

Detection of insertion mutations in exon 12 of *NPM1* and ITDs in *FLT3* was performed by polymerase chain reaction (PCR) as described previously (Gale, *et al* 2008, Thiede, *et al*

2002) using 10 ng of genomic DNA. The PCR products were separated by capillary electrophoresis in an ABI 3130 XL genetic analyzer (Applied Biosystems, Foster City, CA, USA) and fragment sizing was performed using GeneMapper 4.0 software (Applied Biosystems).

Statistical analysis

Associations between genotypes and CR were assessed using logistic regression, adjusting for age. Patient baseline characteristics were compared between genotype groups with Chi² or generalized Fisher's exact test (categorical variables) and Kruskal Wallis test (for age). Kaplan-Meier analysis was used to estimate overall survival (OS) and event free survival (EFS) and the log-rank test to determine significance. Stratification in the survival analysis was performed based on *FLT3* and *NPM1* status to determine the impact of *ABCB1* polymorphisms in patient subgroups. The low frequency genotypes 2677G/A and 2677T/A were excluded in the survival analysis. A p-value of 0.05 was considered significant. Multivariable analysis was performed using the Cox regression model with a forced entry method, and group comparisons between *FLT3*-ITD patients and *FLT3*-wild-type patients were performed based on the Kaplan-Meier curves. Due to the wide time range (1988-2009), year of diagnosis was included as possible confounder in the Cox regression.

Results

Genotyping

All patients were successfully genotyped for the *ABCB1* SNPs 1199G>A, 1236C>T, 2677G>T/A and 3435C>T. The variants were found at the expected frequencies with all genotypes in accordance with the Hardy-Weinberg equation. Genotype frequencies are summarized in **Table II**.

Impact on treatment response and survival

FLT3 and NPM1

As expected, *FLT3*-ITD was significantly associated to a shorter EFS and shorter OS times (median PFS 15 and 32 months for *FLT3*-ITD and *FLT3* wild-type, respectively, $p=0.003$, and median OS 19 and 32 months for *FLT3*-ITD and *FLT3* wild-type, respectively, $p=0.025$). PFS data was missing in 74 patients. No significant independent impact of *NPM1* mutation was seen on EFS or OS, but *FLT3*-ITD/*NPM1* wild-type patients have shorter EFS and shorter OS compared to patients with *NPM1* mutation together with *FLT3* wild-type ($p=0.007$ and $p=0.008$, respectively, data not shown). There was a slightly higher frequency of CR in *NPM1* mutated patients compared to *NPM1* wild-type patients, 89% and 81% CR, respectively, age adjusted OR 1.960, 95%CI 0.854-4.498, $p=0.11$.

ABCB1 SNPs

There was no significant impact of any of the SNPs on the probability of CR (**Table III**), and no overall difference in EFS or OS with regard to *ABCB1* genotypes when the entire material was analyzed. However, when patients were subgrouped according to *FLT3* status, there was an impact on OS of the SNP 1236C>T in *FLT3* wild-type patients ($n=133$) but not in *FLT3*-ITD positive patients; median OS were 20, 58 and 49 months for *FLT3* wild-type 1236C/C, C/T and T/T, respectively, $p=0.059$ (**Figure 1A**). A similar pattern was seen for 2677G>T; median OS was 20, 30 and 49 months for *FLT3* wild-type 2677G/G, G/T and T/T, respectively (**Figure 2A**, $p=0.12$). Comparing patients with at least one variant allele to those with the wild-type alleles strengthened the results; median OS was 20 months in *FLT3* wild-type 1236G/G patients as compared to 49 months in *FLT3* wild-type patients carrying at least one 1236T allele (**Figure 1B**, $p=0.017$), and 20 months in *FLT3* wild-type 2677G/G patients as compared to 35 months in *FLT3* wild-type patients carrying at least one 2677T allele (**Figure 2B**, $p=0.039$). Excluding patients who received allo-SCT did not have a large impact on the survival curves; see **Supplemental data Figure 1-2**. A multivariate Cox regression analysis was used to further investigate the influence of *ABCB1* SNPs 1236C>T and 2677G>T on OS with other factors taken into account. Overall survival without censoring patients at transplantation was analyzed, taking age, gender, *FLT3*-ITD, *NPM1* mutation, treatment (chemotherapy alone or chemotherapy followed by allo-SCT) and *ABCB1* SNP genotype into account. The analysis was adjusted for year of diagnosis due to the wide range (1988-2009), to adjust for minor changes in treatment guidelines over the years. Year of diagnosis as a

continuous variable was used for the results presented below, but adjusting for a categorical grouping variable (pre- or post the introduction of national treatment guidelines) did not alter the results significantly (not shown). Since the SNPs 1236C>T and 2677G>T are present in linkage disequilibrium (Kroetz, *et al* 2003, Marzolini, *et al* 2004), the effect of these SNPs could not be distinguished from each other. Because of this, separate Cox regression analyses were performed for each SNP to determine the influence of the different genotypes on survival. 1236C>T and 2677G>T genotype significantly influenced OS together with age, *FLT3*-ITD/*NPM1* mutation status and treatment (**Table IV**). Grouped analysis was also performed based on *FLT3* status, and confirmed that the effect on OS of the SNPs 1236C>T and 2677G>T was limited to *FLT3* wild-type patients (**Table V**). No significant impact on survival was seen for 3435C>T or 1199G>A, neither in the entire cohort nor in subgroups based on *FLT3* status (for survival curves see **Supplemental data Figure 3 and 4**).

As this study was an extension of a previously published material (Green, *et al* 2012), the influence of these two SNPs on OS was also investigated in the new patients separately (n=105). Similar trends were seen as in the entire material, although the Kaplan Meier curves did not display as clear differences and the log rank tests were not significant. Stratified Cox regression analyses based on *FLT3* status were not performed because of the sample size, which was concluded to be too small to allow inclusion of all the desired variables. For more details see **Supplemental data page 1**.

Discussion

In this study **201** patients with *de novo* NK-AML were analyzed for polymorphisms of *ABCB1* and investigated for correlation to treatment response and patient survival. Our results show that in patients wild-type for *FLT3*, but not in *FLT3*-ITD patients, the SNPs 1236C>T and 2677G>T influence survival with the wild-type C/C and G/G genotypes corresponding to a shorter OS compared to patients with at least one variant allele. The influence on survival of the SNPs, together with age, *FLT3*-ITD/*NPM1* mutation status and treatment, was confirmed by the Cox regression analysis, and a grouped analysis further confirmed that the impact of the *ABCB1* SNPs was limited to *FLT3* wild-type patients. Seedhouse *et al* have previously shown that *ABCB1* SNPs have a relevant influence on gene expression only in cases with

leukemia-specific induction of p-gp expression (Seedhouse, *et al* 2007). Previously published studies also show that AML patients with *FLT3*-ITD are less likely to co-express *ABCB1*, and that there are other underlying biological differences between cells expressing *ABCB1* and cells with *FLT3*-ITD, such as differential expression of the transcriptional regulatory factor FOXO1 (Marzac, *et al* 2006, Seedhouse, *et al* 2014). Taken together, this support our findings that *ABCB1* SNPs may be clinically relevant predominantly in *FLT3* wild-type patients. In addition, it has been shown that increased *ABCB1* expression is a feature associated with higher age, which might explain the weaker association between *ABCB1* SNPs and outcome seen in the second part of our patient cohort (Leith, *et al* 1997). While age as a continuous variable was an independent factor for survival in our material, *FLT3*-ITD/*NPM1* status and allo-SCT appear as stronger predictors for survival independent of age group when using a categorical cut-off of 55 or 60 years in multivariable analysis (data not shown). Thus, the relevant “older age cut-off” for investigating *ABCB1* SNPs in our material was unclear, but studies in a larger cohort of elderly patients only may be warranted.

Our results indicate that *ABCB1* SNPs may be a useful as prognostic markers to distinguish subgroups among the present diverse intermediate risk patient group of *de novo* NK-AML and *FLT3* wild-type genotype. Such tools may contribute to future individualization of the treatment and could subsequently lead to a better outcome for the patient.

Previous studies of *ABCB1* SNPs in different settings have been presented, with inconclusive results. In 2000, Hoffmeyer *et al* reported that presence of the synonymous SNP 3435C>T correlated to a lower intestinal expression of *ABCB1*, a reduced activity of p-glycoprotein and subsequently a higher plasma concentration of the p-glycoprotein substrate digoxin (Hoffmeyer, *et al* 2000). This would point towards the potential of 3435C>T having an impact on treatment response and outcome in patients treated with drugs transported by p-glycoprotein. Such results have been presented by Illmer *et al*, showing a decreased OS and increased risk of relapse in AML patients with the wild-type genotype of 3435C>T (Illmer, *et al* 2002) but we could not confirm this in our material. Our results are supported by Hur *et al*, showing no impact of 3435C>T on leukemic blast p-glycoprotein function or clinical outcome in AML patients (Hur, *et al* 2008).

No other recent studies on the impact of *ABCB1* SNPs on the outcome in AML have been published, but two studies on colorectal cancer patients support our results. Balcerczak et al. demonstrated a positive prognostic effect of the 1236C>T SNP, with 1236T variant carriers having an improved OS (Balcerczak, *et al* 2010). Also, in 2013, De Mattia published a study on colorectal cancer patients treated with the FOLFIRI regimen (including leucovorin, 5-fluorouracil and the topoisomerase inhibitor irinotecan) showing a longer OS for patients with the 2677T-variant, although no correlation to pharmacokinetic profile could be detected (De Mattia, *et al* 2013). In 2007, Kimchi-Sarfaty et al investigated the SNPs 1236C>T, 2677G>T and 3435C>T with the aim of elucidating the effects of synonymous polymorphism on protein expression and transporter function (Kimchi-Sarfaty, *et al* 2007). Their results supported the hypothesis that a change to rare codons would affect co-translational folding and insertion into the cell membrane, thereby affecting substrate binding sites. This also appeared to be of higher importance at higher expression levels of *ABCB1*. Given that the transporter expression is likely to be higher in cancer cells compared to normal cells, the effect of a SNP variant would be more pronounced in the leukemic cell population. We did not have the opportunity to examine *ABCB1* expression in our retrospective study. However, the results by Kimchi-Sarfaty and others, indicating reduced function, expression and/or activity in the variants, could explain the differences in patient outcome seen in our study (Hoffmeyer, *et al* 2000, Kimchi-Sarfaty, *et al* 2007, Tanabe, *et al* 2001). A reduction in drug efflux in leukemic cells would lead to higher intracellular concentrations of the drugs, increasing the anti-tumor effect in patients treated with *ABCB1* substrates. This could subsequently lead to improved survival times. Potentially, a genotyping-phenotyping approach could be an option to identify the patients where *ABCB1* SNPs are most likely to affect treatment with drugs that are *ABCB1* substrates. Since substrate specific differences may be present, further studies in a prospective setting are needed to clarify the predictive value of these SNPs. Also, pharmacogenetics studies of *ABCB1* in connection with clinical trials of drugs that aim to mediate the activity of *ABCB1* would be of interest.

Some limitations of our study needs to be addressed: This is a retrospective study, with a material collected from four different centers, during a long time period, and including

patients with some differences in treatment regimes. However, the inclusion of year of diagnosis in the multivariable analysis would indirectly adjust for minor changes in treatment protocols over the years, and strengthens our results. Also, the age distribution of our study population is largely representative for the AML population subject to induction treatment with curative intent.

Conclusions

In conclusion, we have confirmed our previously published results (Green, *et al* 2012), and our new findings further indicate that the *ABCB1* SNPs 1236C>T and 2677G>T have potential as prognostic markers useful specifically to distinguish relative high risk patients in the diverse intermediate risk patient group of *de novo* NK-AML with *FLT3* wild-type genotype. Further studies on the predictive value are needed, but our results may contribute to better prognostication and future individualization of treatment strategies, and hopefully lead to a better outcome for the future patient.

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Author contributions

IJF: Research, data compilation, statistical analysis, manuscript writing; AF: Data compilation, statistical analysis; EP and MHe: Research, data collection; RR: Data analysis; HN, MHo, LP, DS, and YW: Patient material and data collection; HG: Research, study design, data and statistical analysis; KL: Study design, patient material, and data collection. All authors contributed with critical revision of the manuscript.

Conflicts of interest

The authors have no conflicts of interest to report.

References

- Bacher, U., Haferlach, T., Schoch, C., Kern, W. & Schnittger, S. (2006) Implications of NRAS mutations in AML: a study of 2502 patients. *Blood*, **107**, 3847-3853.
- Balcerczak, E., Panczyk, M., Piaskowski, S., Pasz-Walczak, G., Salagacka, A. & Mirowski, M. (2010) ABCB1/MDR1 gene polymorphisms as a prognostic factor in colorectal cancer. *International Journal of Colorectal Disease*, **25**, 1167-1176.
- Bienz, M., Ludwig, M., Leibundgut, E.O., Mueller, B.U., Ratschiller, D., Solenthaler, M., Fey, M.F. & Pabst, T. (2005) Risk assessment in patients with acute myeloid leukemia and a normal karyotype. *Clinical Cancer Research*, **11**, 1416-1424.
- De Mattia, E., Toffoli, G., Polesel, J., D'Andrea, M., Corona, G., Zagonel, V., Buonadonna, A., Dreussi, E. & Cecchin, E. (2013) Pharmacogenetics of ABC and SLC transporters in metastatic colorectal cancer patients receiving first-line FOLFIRI treatment. *Pharmacogenetics and Genomics*, **23**, 549-557.
- Dohner, H., Estey, E.H., Amadori, S., Appelbaum, F.R., Buchner, T., Burnett, A.K., Dombret, H., Fenaux, P., Grimwade, D., Larson, R.A., Lo-Coco, F., Naoe, T., Niederwieser, D., Ossenkoppele, G.J., Sanz, M.A., Sierra, J., Tallman, M.S., Lowenberg, B. & Bloomfield, C.D. (2010) Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*, **115**, 453-474.
- Evans, W.E. & McLeod, H.L. (2003) Pharmacogenomics--drug disposition, drug targets, and side effects. *New England Journal of Medicine*, **348**, 538-549.
- Falini, B., Mecucci, C., Tiacci, E., Alcalay, M., Rosati, R., Pasqualucci, L., La Starza, R., Diverio, D., Colombo, E., Santucci, A., Bigerna, B., Pacini, R., Pucciarini, A., Liso, A., Vignetti, M., Fazi, P., Meani, N., Pettirossi, V., Saglio, G., Mandelli, F., Lo-Coco, F., Pelicci, P.G. & Martelli, M.F. (2005) Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *New England Journal of Medicine*, **352**, 254-266.
- Falk, I.J., Fyrberg, A., Paul, E., Nahi, H., Hermanson, M., Rosenquist, R., Hoglund, M., Palmqvist, L., Stockelberg, D., Wei, Y., Green, H. & Lotfi, K. (2013) Decreased survival in normal karyotype AML with single-nucleotide polymorphisms in genes encoding the AraC metabolizing enzymes cytidine deaminase and 5'-nucleotidase. *American Journal of Hematology*, **88**, 1001-1006.
- Gale, R.E., Green, C., Allen, C., Mead, A.J., Burnett, A.K., Hills, R.K. & Linch, D.C. (2008) The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. *Blood*, **111**, 2776-2784.
- Germann, U.A. (1996) P-glycoprotein--a mediator of multidrug resistance in tumour cells. *European Journal of Cancer*, **32A**, 927-944.
- Green, H., Falk, I.J., Lotfi, K., Paul, E., Hermansson, M., Rosenquist, R., Paul, C. & Nahi, H. (2012) Association of ABCB1 polymorphisms with survival and in vitro cytotoxicity in de novo acute myeloid leukemia with normal karyotype. *Pharmacogenomics Journal*, **12**, 111-118.
- Green, H., Soderkvist, P., Rosenberg, P., Horvath, G. & Peterson, C. (2006) mdr-1 single nucleotide polymorphisms in ovarian cancer tissue: G2677T/A correlates with response to paclitaxel chemotherapy. *Clinical Cancer Research*, **12**, 854-859.

- Green, H., Soderkvist, P., Rosenberg, P., Horvath, G. & Peterson, C. (2008) ABCB1 G1199A polymorphism and ovarian cancer response to paclitaxel. *Journal of Pharmaceutical Sciences*, **97**, 2045-2048.
- Grimwade, D., Hills, R.K., Moorman, A.V., Walker, H., Chatters, S., Goldstone, A.H., Wheatley, K., Harrison, C.J. & Burnett, A.K. (2010) Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*, **116**, 354-365.
- Grimwade, D., Walker, H., Harrison, G., Oliver, F., Chatters, S., Harrison, C.J., Wheatley, K., Burnett, A.K. & Goldstone, A.H. (2001) The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. *Blood*, **98**, 1312-1320.
- Hoffmeyer, S., Burk, O., von Richter, O., Arnold, H.P., Brockmoller, J., John, A., Cascorbi, I., Gerloff, T., Roots, I., Eichelbaum, M. & Brinkmann, U. (2000) Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 3473-3478.
- Hur, E.H., Lee, J.H., Lee, M.J., Choi, S.J., Kang, M.J., Seol, M., Jang, Y.E., Lee, H.J., Kang, I.S., Shim, S.K., Ryu, S.G., Kang, Y.A., Lee, Y.S., Park, C.J., Chi, H.S. & Lee, K.H. (2008) C3435T polymorphism of the MDR1 gene is not associated with P-glycoprotein function of leukemic blasts and clinical outcome in patients with acute myeloid leukemia. *Leukemia Research*, **32**, 1601-1604.
- Illmer, T., Schuler, U.S., Thiede, C., Schwarz, U.I., Kim, R.B., Gotthard, S., Freund, D., Schakel, U., Ehninger, G. & Schaich, M. (2002) MDR1 gene polymorphisms affect therapy outcome in acute myeloid leukemia patients. *Cancer Research*, **62**, 4955-4962.
- Juliusson, G., Antunovic, P., Derolf, A., Lehmann, S., Mollgard, L., Stockelberg, D., Tidefelt, U., Wahlin, A. & Hoglund, M. (2009) Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood*, **113**, 4179-4187.
- Kim, R.B., Leake, B.F., Choo, E.F., Dresser, G.K., Kubba, S.V., Schwarz, U.I., Taylor, A., Xie, H.G., McKinsey, J., Zhou, S., Lan, L.B., Schuetz, J.D., Schuetz, E.G. & Wilkinson, G.R. (2001) Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clinical Pharmacology and Therapeutics*, **70**, 189-199.
- Kimchi-Sarfaty, C., Oh, J.M., Kim, I.W., Sauna, Z.E., Calcagno, A.M., Ambudkar, S.V. & Gottesman, M.M. (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science*, **315**, 525-528.
- Kroetz, D.L., Pauli-Magnus, C., Hodges, L.M., Huang, C.C., Kawamoto, M., Johns, S.J., Stryke, D., Ferrin, T.E., DeYoung, J., Taylor, T., Carlson, E.J., Herskowitz, I., Giacomini, K.M. & Clark, A.G. (2003) Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. *Pharmacogenetics*, **13**, 481-494.
- Leith, C.P., Kopecky, K.J., Godwin, J., McConnell, T., Slovak, M.L., Chen, I.M., Head, D.R., Appelbaum, F.R. & Willman, C.L. (1997) Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. *Blood*, **89**, 3323-3329.
- Marzac, C., Teyssandier, I., Calendini, O., Perrot, J.Y., Faussat, A.M., Tang, R., Casadevall, N., Marie, J.P. & Legrand, O. (2006) FLT3 internal tandem duplication and P-glycoprotein functionality in 171 patients with acute myeloid leukemia. *Clinical Cancer Research*, **12**, 7018-7024.
- Marzolini, C., Paus, E., Buclin, T. & Kim, R.B. (2004) Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clinical Pharmacology and Therapeutics*, **75**, 13-33.

- Mrozek, K., Marcucci, G., Paschka, P., Whitman, S.P. & Bloomfield, C.D. (2007) Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? *Blood*, **109**, 431-448.
- Nakao, M., Yokota, S., Iwai, T., Kaneko, H., Horiike, S., Kashima, K., Sonoda, Y., Fujimoto, T. & Misawa, S. (1996) Internal tandem duplication of the *flt3* gene found in acute myeloid leukemia. *Leukemia*, **10**, 1911-1918.
- Renneville, A., Boissel, N., Gachard, N., Naguib, D., Bastard, C., de Botton, S., Nibourel, O., Pautas, C., Reman, O., Thomas, X., Gardin, C., Terre, C., Castaigne, S., Preudhomme, C. & Dombret, H. (2009) The favorable impact of CEBPA mutations in patients with acute myeloid leukemia is only observed in the absence of associated cytogenetic abnormalities and FLT3 internal duplication. *Blood*, **113**, 5090-5093.
- Robert, J., Morvan, V.L., Smith, D., Pourquier, P. & Bonnet, J. (2005) Predicting drug response and toxicity based on gene polymorphisms. *Critical Reviews in Oncology/Hematology*, **54**, 171-196.
- Seedhouse, C.H., Grundy, M., White, P., Li, Y., Fisher, J., Yakunina, D., Moorman, A.V., Hoy, T., Russell, N., Burnett, A. & Pallis, M. (2007) Sequential influences of leukemia-specific and genetic factors on p-glycoprotein expression in blasts from 817 patients entered into the National Cancer Research Network acute myeloid leukemia 14 and 15 trials. *Clinical Cancer Research*, **13**, 7059-7066.
- Seedhouse, C.H., Mills, K.I., Ahluwalia, S., Grundy, M., Shang, S., Burnett, A.K., Russell, N.H. & Pallis, M. (2014) Distinct poor prognostic subgroups of acute myeloid leukaemia, FLT3-ITD and P-glycoprotein-positive, have contrasting levels of FOXO1. *Leukemia Research*, **38**, 131-137.
- Tanabe, M., Ieiri, I., Nagata, N., Inoue, K., Ito, S., Kanamori, Y., Takahashi, M., Kurata, Y., Kigawa, J., Higuchi, S., Terakawa, N. & Otsubo, K. (2001) Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *The Journal of Pharmacology and Experimental Therapeutics*, **297**, 1137-1143.
- Thiede, C., Steudel, C., Mohr, B., Schaich, M., Schakel, U., Platzbecker, U., Wermke, M., Bornhauser, M., Ritter, M., Neubauer, A., Ehninger, G. & Illmer, T. (2002) Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood*, **99**, 4326-4335.
- Wahlin, A., Billstrom, R., Bjor, O., Ahlgren, T., Hedenus, M., Hoglund, M., Lindmark, A., Markevarn, B., Nilsson, B., Sallerfors, B. & Brune, M. (2009) Results of risk-adapted therapy in acute myeloid leukaemia. A long-term population-based follow-up study. *European Journal of Haematology*, **83**, 99-107.
- van den Heuvel-Eibrink, M.M., Wiemer, E.A., de Boevere, M.J., van der Holt, B., Vossebeld, P.J., Pieters, R. & Sonneveld, P. (2001) MDR1 gene-related clonal selection and P-glycoprotein function and expression in relapsed or refractory acute myeloid leukemia. *Blood*, **97**, 3605-3611.
- van der Holt, B., Van den Heuvel-Eibrink, M.M., Van Schaik, R.H., van der Heiden, I.P., Wiemer, E.A., Vossebeld, P.J., Lowenberg, B. & Sonneveld, P. (2006) ABCB1 gene polymorphisms are not associated with treatment outcome in elderly acute myeloid leukemia patients. *Clinical Pharmacology and Therapeutics*, **80**, 427-439.

Table I. NK-AML patient characteristics.

<i>AML patient characteristics</i>		<i>Total N =201</i>
<i>Gender</i>		
	Male	95 (47%)
	Female	106 (53%)
<i>Age at diagnosis, mean (range)</i>		59 (18–85)
<i>FLT3 status</i>		
	<i>FLT3</i> wild type	133 (66%)
	<i>FLT3</i> internal tandem duplication	66 (33%)
	Missing information	2 (1%)
<i>NPM1 status</i>		
	<i>NPM1</i> wild type	108 (54%)
	<i>NPM1</i> mutated	91 (45%)
	Missing information	2 (1%)
<i>Treatment^a</i>		
	Dnr + AraC	122 (59.5%)
	Ida + AraC or Ida + AraC + Eto	42 (20.5%)
	Ida + AraC + CdA	12 (6%)
	High dose AraC	5 (2.5%)
	Mitox + AraC or Mitox + AraC + Eto	9 (4%)
	Dnr + AraC + 6-TG	8 (4%)
	Other	3 (1.5%)
<i>Treatment response^b</i>		
	CR	168 (83.5%)
	Non-CR	32 (16%)
	Missing [*]	1 (0.5%)

^aDnr = Daunorubicine; AraC = Cytarabine; Ida = Idarubicine; Eto = etoposide; CdA = Cladribine; Mitox = Mitoxantrone; 6-TG = 6-thioguanine; Other= including one patient without known treatment details but curative intent reported, one with Fludarabine + Granulocyte-colony stimulating factor + AraC, and one with AraC + amekrin. ^bCR = complete remission. ^{*}No details reported.

Table II. *ABCB1* genotype frequencies in 201 NK-AML patients.

SNP	Genotype	N	Freq. (%)
1199G>A (<i>rs2229109</i>)	G/G	185	92.0
	G/A	16	8.0
1236C>T (<i>rs1128503</i>)	C/C	65	32.3
	C/T	98	48.8
	T/T	38	18.9
2677G>T/A (<i>rs2032582</i>)	G/G	62	30.8
	G/T	93	46.3
	T/T	38	18.9
	G/A	4	2.0
	A/T	4	2.0
3435C>T (<i>rs1045642</i>)	C/C	38	18.9
	C/T	98	48.8
	T/T	65	32.3

Table III. *ABCB1* genotype distributions in relation to treatment response.
Response data was missing in 1 patient. Age adjusted logistic regression.

SNP	CR	No CR	OR (95% CI)	p
1199G>A				
G/G	156	28	1	
G/A	12	4	0.596 (0.175-2.031)	0.408
1236C>T				
C/C	52	13	1	
C/T	83	14	1.459 (0.623-3.416)	0.384
T/T	33	5	2.021 (0.637-6.413)	0.232
2677G>T				
G/G	50	12	1	
G/T	78	14	1.374 (0.577-3.274)	0.473
T/T	32	6	1.428 (0.474-4.308)	0.527
3435C>T				
C/C	33	5	1	
C/T	81	16	0.815 (0.270-2.464)	0.717
T/T	54	11	0.800 (0.249-2.566)	0.707

Table IV. Cox regression analysis of OS. The regression analysis were split into two models since the SNPs 1236C>T and 2677G>T are so closely linked that the effect of one SNP could not be distinguished from the other. The analysis was adjusted for year of diagnosis due to the wide range (1988-2009).

Model with 1236C>T – Unstratified (n=201)				Model with 2677G>T* – Unstratified (n=201)			
Variable	<i>p</i>	HR	(95% CI)	Variable	<i>p</i>	HR	(95% CI)
Age at diag.	0.025	1.024	(1.003-1.045)	Age at diag.	0.005	1.031	(1.009-1.054)
Gender ¹	0.740	0.933	(0.621-1.403)	Gender ¹	0.705	1.069	(0.609-1.399)
<i>FLT3</i> -ITD-/NPM1+		1		<i>FLT3</i> -ITD-/NPM1+			
<i>FLT3</i> -ITD+/NPM1+	0.066	1.846	(0.959-3.551)	<i>FLT3</i> -ITD+/NPM1+	0.141	1.661	(0.845-3.264)
<i>FLT3</i> -ITD+/NPM1-	0.003	2.884	(1.427-5.826)	<i>FLT3</i> -ITD+/NPM1-	0.013	2.514	(1.215-5.204)
<i>FLT3</i> -ITD-/NPM1-	0.152	1.508	(0.860-2.642)	<i>FLT3</i> -ITD-/NPM1-	0.197	1.463	(0.821-2.609)
Chemotherapy only		1		Chemotherapy only		1	
Chemotherapy + allo-SCT	0.002	0.379	(0.203-0.708)	Chemotherapy + allo-SCT	0.016	0.450	(0.235-0.861)
1236 C/C		1		2677 G/G		1	
1236 C/T	0.012	0.562	(0.359-0.880)	2677 G/T	0.008	0.531	(0.333-0.846)
1236 T/T	0.020	0.493	(0.273-0.893)	2677 T/T	0.015	0.464	(0.250-0.862)

* - For G2677T/A the A-allele was excluded from the analysis due to low frequency. HR – Hazard ratio, 95% CI – 95% confidence interval for the hazard ratio. ¹ Female compared to male gender

Table V. Cox regression analysis of OS, grouped analysis based on *FLT3* status. The regression analysis were split into two models since the SNPs 1236C>T and 2677G>T are so closely linked that the effect of one SNP could not be distinguished from the other. The analysis was adjusted for year of diagnosis due to the wide range (1988-2009).

Model with 1236C>T – <i>FLT3</i> wild-type patients (n=133)				Model with 2677G>T* – <i>FLT3</i> wild-type patients (n=133)			
Variable	P=	HR	(95% CI)	Variable	P=	HR	(95% CI)
Age at diag.	0.007	1.035	(1.010-1.062)	Age at diag.	0.001	1.045	(1.018-1.074)
Gender ¹	0.155	1.485	(0.861-2.561)	Gender ¹	0.222	1.415	(0.811-2.471)
<i>NPM1</i> mutation	0.212	0.693	(0.390-1.232)	<i>NPM1</i> mutation	0.254	0.705	(0.387-1.285)
Chemotherapy only		1		Chemotherapy only		1	
Chemotherapy + allo-SCT	0.045	0.415	(0.176-0.980)	Chemotherapy + allo-SCT	0.125	0.497	(0.203-1.285)
1236 C/C		1		2677 G/G		1	
1236 C/T	0.021	0.499	(0.276-0.901)	2677 G/T	0.020	0.465	(0.244-0.887)
1236 T/T	0.009	0.402	(0.203-0.798)	2677 T/T	0.007	0.368	(0.178-0.761)
Model with 1236C>T – <i>FLT3</i> -ITD patients (n=66)				Model with 2677G>T* – <i>FLT3</i> -ITD patients (n=66)			
Variable	P=	HR	(95% CI)	Variable	P=	HR	(95% CI)
Age at diag.	0.703	0.992	(0.953-1.033)	Age at diag.	0.892	0.997	(0.957-1.039)
Gender ¹	0.398	0.725	(0.344-1.528)	Gender ¹	0.430	0.725	(0.327-1.610)
<i>NPM1</i> mutation	0.068	0.506	(0.243-1.052)	<i>NPM1</i> mutation	0.086	0.510	(0.237-1.100)
Chemotherapy only		1		Chemotherapy only		1	
Chemotherapy + allo-SCT	0.025	0.286	(0.096-0.853)	Chemotherapy + allo-SCT	0.052	0.332	(0.109-1.009)
1236 C/C		1		2677 G/G		1	
1236 C/T	0.811	0.907	(0.407-2.022)	2677 G/T	0.623	0.822	(0.377-1.792)
1236 T/T	0.648	0.752	(0.221-2.555)	2677 T/T	0.506	0.640	(0.172-2.384)

* - For G2677T/A the A-allele was excluded from the analysis due to low frequency. HR – Hazard ratio, 95% CI – 95% confidence interval for the hazard ratio. ¹) Female compared to male gender.

Figure legends

Figure 1. Impact of *ABCB1* SNP 1236C>T on OS in *FLT3* wild-type patients. **(A)** Median OS 20, 58 and 49 months for *FLT3* wild-type 1236C/C, C/T and T/T, respectively, $p=0.059$. **(B)** *FLT3* wild-type 1236C/C patients have a significantly shorter OS compared to patients carrying at least one variant allele; median OS 20 vs. 49 months, respectively, $p=0.017$.

Figure 2. Impact of *ABCB1* SNP 2677G>T (low frequency A-allele excluded) on OS in *FLT3* wild-type patients. **(A)** Median OS 20, 30 and 49 months for *FLT3* wild-type 2677G/G, G/T and T/T, respectively, $p=0.12$. **(B)** *FLT3* wild-type 2677G/G patients have a significantly shorter OS compared to patients carrying at least one variant allele; median 20 vs. 35 months, respectively, $p=0.039$.

1B

2A

2B

Supplemental data

As this study was an extension of a previously published material [18], the influence of these two SNPs on OS was also investigated in the new patients separately (n=105).

In *FLT3* wild-type patients (n=69), mean OS was 28 months for patients with the 1236C/C genotype compared to 70 months for patients with at least one T-allele (median not reached for C/T). The same was seen in *FLT3* wild-type patients for the SNP 2677G>T; mean OS 29 months for patients with the 2677G/G genotype compared to 70 months for patients with at least one T-allele. The Kaplan Meier curves did not display as clear differences as for the entire material and the log rank tests were not significant.

In addition, Cox regression analyses of OS were performed for the previously unpublished 105 patients, and showed similar patterns as for the previously published patients, as well as for the entire study material. Compared to 1236C/C patients, hazard ratios (HR) was 0.894 (95%CI 0.448-1.782) and 0.561 (95%CI 0.214-1.470) for 1236C/T and 1236T/T patients, respectively. For the 2677G>T SNP, HR was 0.914 (95%CI 0.462-1.809) for G/T patients and 0.517 (95%CI 0.184-1.449) for T/T patients, compared to the 2677G/G patients. This is also in line with the previously published material as well as the results for the entire cohort, but for neither of the SNPs the results were statistically significant. Stratified Cox regression analyses based on *FLT3* status were not performed because of the sample size, which was concluded to be too small to allow inclusion of all the desired variables. It should be noted that the new patients were significantly younger at diagnosis compared to the patients from the previously published study (median age 59 and 65 years, respectively, p=0.02).

Survival analysis, excluding patients with allo-SCT.

1A

1B

Figure 1A. Impact of *ABCB1* SNP 1236C>T on OS in *FLT3* wild-type patients, excluding those with allo-SCT. **(A)** Median OS 15, 32 and 35 months for *FLT3* wild-type 1236C/C, C/T and T/T, respectively,

p=0.075. **(B)** *FLT3* wild-type 1236C/C patients have a significantly shorter OS compared to patients carrying at least one variant allele; median OS 15 and 35 months, respectively, p=0.024.

2A.

2B.

Figure 2. Impact of *ABCB1* SNP 2677G>T (low frequency A-allele excluded) on OS in *FLT3* wild-type patients, excluding those with allo-SCT. **(A)** Median OS 20, 30 and 49 months for *FLT3* wild-type

2677G/G, G/T and T/T, respectively, $p=0.20$. **(B)** *FLT3* wild-type 2677G/G patients have a significantly shorter OS compared to patients carrying at least one variant allele; median 20 and 32 months, respectively, $p=0.074$.

Cox regressions, excluding patients treated with allo-SCT.

Table I. All patients, excluding allo-SCT. The analysis was adjusted for year of diagnosis due to the wide range (1992-2009).

Model with 1236C>T – Unstratified (n=144)				Model with 2677G>T* – Unstratified (n=144)			
Variable	<i>p</i>	HR	(95% CI)	Variable	<i>p</i>	HR	(95% CI)
Age at diag.	0.020	1.031	(1.005-1.057)	Age at diag.	0.003	1.041	(1.014-1.069)
Gender ¹	0.367	0.810	(0.511-1.282)	Gender ¹	0.391	0.814	(0.508-1.303)
<i>FLT3</i> -ITD-/NPM1+		1		<i>FLT3</i> -ITD-/NPM1+		1	
<i>FLT3</i> -ITD+/NPM1+	0.063	2.010	(0.964-4.191)	<i>FLT3</i> -ITD+/NPM1+	0.133	1.781	(0.838-3.784)
<i>FLT3</i> -ITD+/NPM1-	0.007	2.878	(1.333-6.210)	<i>FLT3</i> -ITD+/NPM1-	0.024	2.547	(1.133-5.726)
<i>FLT3</i> -ITD-/NPM1-	0.214	1.482	(0.797-2.755)	<i>FLT3</i> -ITD-/NPM1-	0.242	1.462	(0.774-2.759)
1236 C/C		1		2677 G/G		1	
1236 C/T	0.016	0.525	(0.311-0.888)	2677 G/T	0.029	0.555	(0.326-0.942)
1236 T/T	0.017	0.458	(0.241-0.871)	2677 T/T	0.024	0.463	(0.282-0.972)

* - For G2677T/A the A-allele was excluded from the analysis due to low frequency. HR – Hazard ratio, 95% CI – 95% confidence interval for the hazard ratio. ¹) Female compared to male gender.

Table II. Grouped analysis based on *FLT3* status, excluding allo-SCT. The analysis was adjusted for year of diagnosis due to the wide range (1992-2009).

Model with 1236C>T – <i>FLT3</i> wild-type patients (n=100)				Model with 2677G>T* – <i>FLT3</i> wild-type patients (n=100)			
Variable	P=	HR	(95% CI)	Variable	P=	HR	(95% CI)
Age at diag.	0.009	1.040	(1.010-1.072)	Age at diag.	0.001	1.053	(1.020-1.086)
Gender ¹	0.534	0.832	(0.467-1.485)	Gender ¹	0.663	0.878	(0.489-1.577)
<i>NPM1</i> mutation	0.147	0.620	(0.325-1.184)	<i>NPM1</i> mutation	0.183	0.638	(0.330-1.236)
1236 C/C		1		2677 G/G		1	
1236 C/T	0.023	0.450	(0.226-0.898)	2677 G/T	0.042	0.488	(0.245-0.974)
1236 T/T	0.006	0.345	(0.161-0.736)	2677 T/T	0.007	0.340	(0.154-0.750)
Model with 1236C>T – <i>FLT3</i> -ITD patients (n=42)				Model with 2677G>T* – <i>FLT3</i> -ITD patients (n=42)			
Variable	P=	HR	(95% CI)	Variable	P=	HR	(95% CI)

Age at diag.	0.884	0.996	(0.943-1.052)	Age at diag.	0.864	1.005	(0.950-1.063)
Gender ¹	0.721	1.169	(0.496-2.760)	Gender ¹	0.741	1.179	(0.449-3.077)
<i>NPM1</i> mutation	0.168	0.534	(0.219-1.302)	<i>NPM1</i> mutation	0.176	0.531	(0.212-1.328)
1236 C/C		1		2677 G/G		1	
1236 C/T	0.761	0.853	(0.306-2.379)	2677 G/T	0.843	0.904	(0.335-2.440)
1236 T/T	0.872	0.892	(0.222-3.583)	2677 T/T	0.952	0.957	(0.229-3.992)

* - For G2677T/A the A-allele was excluded from the analysis due to low frequency. HR – Hazard ratio, 95% CI – 95% confidence interval for the hazard ratio. ¹⁾ Female compared to male gender.

Survival curves for 3435C>T and 1199G>A.

No significant influence of 3435C>T on overall survival was seen in the entire cohort (**Figure 3A**) or in *FLT3* subsets (**3B-C**). No significant influence on overall survival was seen for 1199G>A (**Figure 4**); variant allele frequency was determined to be too low for further sub group analysis.

Figure 3. (A) OS 63, 36 and 38 months for 3435C/C, C/T and T/T, respectively; $p=0.551$. **(B)** OS in FLT3 wild type patients was 60, 40 and 44 months for 3435C/C, C/T and T/T, respectively; $p=0.702$. **(C)** OS in FLT3-ITD positive patients was 51, 18 and 22 months for 3435C/C, C/T and T/T, respectively; $p=0.322$.

Figure 4. OS was 45 and 17 months for 1199G/G and G/A patients, respectively; p=0.241.