Published in final edited form as:
Cancer. 2014 February 15; 120(4): 521–529. doi:10.1002/cncr.28368.

Low 25(OH) Vitamin D_3 Levels Are Associated with Adverse Outcome in Newly-Diagnosed Intensively-Treated Adult Acute Myeloid Leukemia Patients

Hun Ju Lee, MD[1,*], **Josephia R. Muindi, MD, PhD**[2], **Wei Tan, MA**[3], **Qiang Hu, PhD**[3], **Dan Wang, MA**[3], **Song Liu, PhD**[3], **Gregory E. Wilding, PhD**[3], **Laurie A. Ford, BS**[1], **Sheila N.J. Sait, PhD**[4], **Annemarie W. Block, PhD**[4], **Araba A. Adjei, PhD**[2], **Maurice Barcos, MD, PhD**[5], **Elizabeth A Griffiths, MD**[1], **James E. Thompson, MD**[1], **Eunice S. Wang, MD**[1], **Candace S Johnson, PhD**[2], **Donald L. Trump, MD**[6], and **Meir Wetzler, MD**[1]

[1]Leukemia Section, Department of Medicine, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263 USA

[2]Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263 USA

[3]Department of Biostatistics, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263 USA

[4]Clinical Cytogenetics Laboratory, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263 USA

[5]Department of Pathology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263 USA

[6]Department of Medicine, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263 USA

Corresponding Author: Meir Wetzler, MD, Leukemia Section, Department of Medicine, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY, 14263, USA, Tel: 716-845-8447, Fax: 716-845-2343, meir.wetzler@roswellpark.org.
[*]Current Address: Department of Lymphoma & Myeloma, M.D. Anderson Cancer Center, Houston, TX

Contributions:
H.J. Lee analyzed the data and wrote the manuscript.
J. Muindi analyzed vitamin D levels.
W. Tan performed the statistical analyses in relation to outcome.
Q. Hu assisted in the analysis of the miRs and SNPs databases.
D. Wang assisted in the analysis of the miRs and SNPs databases
S. Liu oversaw the statistical analysis of the miRs and SNPs databases.
G.E. Wilding oversaw the statistical analyses in relation to outcome.
L.A. Ford created the patient database.
S.N.J. Sait analyzed the patients' karyotypes.
A.W. Block analyzed the patients' karyotypes.
A.A. Adjei contributed the SNPs and assisted in data analysis.
M. Barcos oversaw the diagnosis of the patients.
E.A Griffiths contributed to the care of the patients.
J.E. Thompson, MD contributed to the care of the patients.
E.S. Wang contributed to the care of the patients.
C.S Johnson assisted in data analysis.
D.L. Trump asked the question about the role of vitamin D in AML.
M. Wetzler oversaw the conduct of the study and manuscript preparation and contributed to the care of the patients.
All authors reviewed and approved the final manuscript.

CONFLICT OF INTEREST
The authors have no conflict of interest.

Abstract

Background—Several studies suggest that low 25(OH) vitamin D_3 levels may be prognostic in some malignancies, but no studies have evaluated their impact on treatment outcome in acute myeloid leukemia (AML).

Methods—VD levels were evaluated in 97 consecutive newly diagnosed, intensively-treated AML patients. MicroRNA-expression profiles and single nucleotide polymorphisms (SNPs) in the 25(OH) vitamin D_3 pathway genes were evaluated and correlated with 25(OH) vitamin D_3 levels and treatment outcome.

Results—Thirty-four (35%) patients had normal 25(OH) vitamin D_3 levels (32–100 ng/ml), 34 (35%) insufficient (20–31.9 ng/ml) and 29 (30%) deficient levels (<20 ng/ml). Insufficient/deficient 25(OH) vitamin D_3 levels were associated with worse relapse-free survival (RFS) compared to normal vitamin D_3 levels. In multivariate analyses, deficient 25(OH) vitamin D_3, smoking, European LeukemiaNet Genetic Groups and white blood cell count retained their statistical significance for RFS. A number of microRNAs and SNPs were found to be associated with 25(OH) vitamin D_3 level, although none remained significant after multiple test corrections; one 25(OH) vitamin D_3 receptor SNP, rs10783219, was associated with lower complete remission rate (p=0.0442), shorter RFS (p=0.0058) and overall survival (p=0.0011).

Conclusions—It remains to be determined what role microRNA and SNP profiles play in contributing to low 25(OH) vitamin D_3 level and/or outcome and whether supplementation will improve AML outcome.

INTRODUCTION

Epidemiologic studies suggest an association between low 25(OH) vitamin D_3 level and acute myeloid leukemia (AML). For example, a study from the United Arab Emirates (UAE) [1] found that AML is more common among adult females than among adult males, despite the fact that the population of the UAE consists of more males than females and that AML is widely known to be more common in males. These findings suggested that low vitamin D_3 levels, secondary to the practice of women wearing extensive body coverage [2], may contribute to the higher incidence of AML.

In addition, vitamin D was shown in the early 1980s to differentiate AML cells into mature myeloid cells [3]. Therefore, it would suggest that low serum 25(OH) vitamin D_3 levels might be associated with enhanced clonal proliferation. Interestingly, low serum 25(OH) vitamin D_3 levels were associated with inferior event-free survival and overall survival (OS) in diffuse large B- and T-cell non-Hodgkin lymphoma (NHL) [4] and vitamin D insufficiency at diagnosis was associated with decreased time until initiation of treatment in chronic lymphocytic leukemia (CLL) [5]. We therefore hypothesized that vitamin D level at diagnosis be associated with outcome in intensively-treated AML patients.

Vitamin D predominantly exerts its effects through binding to the cognate nuclear vitamin D receptor (VDR); ligand bound VDR heterodimerizes with the retinoic X receptor (RXR) and binds to vitamin D responsive elements in the promoter regions of target genes, such as *CYP24A1*, *BGLAP* (osteocalcin) and cyclin dependent kinase inhibitor 1A (*CDKN1A*, $p21^{Waf1/Cip1}$), several protein kinase C (*PKC*) isoforms [6], the p42 extracellular regulated kinase (p42 *ERK*) and c-Jun N-terminal kinases (*JNK*) families of mitogen activated protein kinases (*MAPKs*) which are important in differentiation, metabolism and cell cycle [7]. We have recently shown that one of the small non-coding RNAs (microRNA, miR), key controllers of cellular function, miR106b, increases in response to vitamin D exposure [8]. We therefore also hypothesized that miR expression will differ among AML patients with low vitamin D levels as compared to those with normal vitamin D levels.

Finally, it has been hypothesized that for individuals with similar vitamin D intake or status, those with VDR or other vitamin D pathway polymorphisms may have increased susceptibility to colo-rectal cancer risk. However, evidence to date is inconclusive. Phenotypic variations such as this, are often due to genetic variations of polymorphic genes encoding the proteins that biotransform Vitamin D leading to variations in serum 25(OH) Vitamin D_3 levels [9]. A recent meta-analysis [10] of relevant vitamin D studies demonstrated an inverse association between vitamin D intake, vitamin D status and the *Bmsl* VDR polymorphism (rs1544410) and colo-rectal cancer risk. Therefore, we included the hypothesis that single nucleotide polymorphisms (SNP) in the vitamin D pathway genes may play a role in AML.

METHODS

Patients and Treatment

Pretreatment bone marrow, peripheral blood and serum were obtained from 97 AML (excluding acute promyelocytic leukemia) patients, 19–91 (median 60) years of age, who received intensive first-line therapy with ADE [cytarabine (100 mg/m^2/day×7 days), daunorubicin (90 mg/m^2/day×3 days for patients <60 years of age and 60 mg/m^2/day×3 days for patients ≥60 years of age) and etoposide (100 mg/m^2/day×3 days)]. Thirty patients in complete remission (CR) received consolidation with high-dose cytarabine; eight received ADE (for five, two and two days of the same doses) and the others received miscellaneous regimens as consolidation. Seven proceeded to an autologous stem cell transplant (SCT) and 16 to an allogeneic SCT in first complete remission (CR). All patients provided informed consent to treatment, sample procurement and further testing; treatments were in accordance with the Declaration of Helsinki and approved by Roswell Park Cancer Institute (RPCI) institutional review board. The RPCI Scientific Review Committee and IRB approved this study.

25(OH) Vitamin D_3 Levels

Serum 25(OH) vitamin D_3 levels were analyzed by a standard commercially available 25-Hydroxyvitamin D_3-[I^{125}] RIA kit from DiaSorin Co. (Stillwater, MN) [11]. The lower limit of normal for this assay is 32 ng/ml, which is based on maximum suppression of parathyroid hormone;[12] the normal range is 32–100 ng/ml (80–250 nmol/mL), insufficient levels were 20–31.9 ng/ml and deficient levels were <20 ng/ml.[13] Samples from the healthy volunteers were assayed in the laboratory of Dr. Bruce W. Hollis using the same radioimmunoassay [12]. Serum 25(OH) vitamin D_3 measurements and normal ranges in both laboratories were the same.

MiR Profiling

An exploratory analysis of 20 samples [10 with subnormal (<32 ng/ml) and 10 with normal or above normal vitamin 25(OH) D_3 levels] was performed on miR arrays by our core facility using the Exiqon platform (Woburn, MA). Samples were labeled with Cy3. Up-regulation of 14 miRs and down-regulation of three miRs were detected. Additional 58 samples were studied by reverse transcriptase polymerase chain reaction (RT-PCR) for the 16 miRs (one miR sequence was not available for study).

TagSNP Selection and Genotyping

TagSNPs were derived from vitamin D metabolism pathway genes including four cytochrome P450 family members *CYP24A1*, *CYP27A1*, *CYP27B1* and *CYP2R1* responsible for vitamin D metabolism (hydroxylation), *GC*, the group-specific component (vitamin D binding protein) for transport, and *VDR* as the target of vitamin D. Briefly, SNP genotype

datasets for Caucasians were selected from the National Center for Biotechnology Information and HapMap databases, in addition to resequencing genotype data for *CYP24A1* and *GC*, that was generated similarly to previous reports [14], using Caucasian DNA samples from the Coriell Cell Repository (Camden NJ, USA). The genotype data were then loaded into the Haploview program to derive both haplotype- and linkage disequilibrium (LD)-tagSNPs for the study [15]. A total of 90 tagSNPs were genotyped on the Sequenom MassARRAY platform (San Diego, CA) by our core facility in accordance with the manufacturer's instructions. Controls were included to ensure genotyping accuracy in addition to genotyping approximately 10% of the samples in duplicates.

Statistical Analyses

Outcome analyses—CR, relapse-free survival (RFS) and OS were defined as previously described [16]. Descriptive statistics such as frequencies and relative frequencies were computed for categorical variables. Numeric variables were summarized using simple descriptive statistics such as the mean, standard deviation, median, range, etc. Fisher's exact test was used to study the association between categorical variables. The Wilcoxon rank sum test was used to compare the groups in regards to numeric variables. Estimation of OS and RFS distributions were done using the Kaplan-Meier method. Patients alive at last follow-up were censored. Using this distributed estimate, summary descriptive statistics such as the median survival and 95% confidence interval (CI) of the median survival were obtained. Statistical assessment of observed differences in the survival distributions of different groups of interest was done using the log-rank test. Cox proportional hazards model was used to assess the effect of study variables on survival for both univariate and multivariate analyses. The Cox Hazard Ratio is a standard assessment of the incremental increase in the odds of patients having a given outcome. A logistic regression model was used to investigate the association between CR statuses and vitamin D groups. The computation of p-value and the 95% confidence interval for odds ratio was based on Wald test. A 0.05 nominal significance level was used in all testing. All statistical analyses were done using SAS (version 9.3).

MiR and SNP data analyses—All data analysis was performed under R programming environment (www.r-project.org). For miR analysis, we used the t-test to calculate the level of differential miR expression between subnormal (<32 ng/ml) and normal (≥32 ng/ml) 25(OH) vitamin D_3 levels. For SNP analysis, we first treated the patients' 25(OH) vitamin D_3 level as continuous variable, and used linear regression to examine whether the SNPs genotypes were significantly associated with the patients' 25(OH) vitamin D_3 level. Then, we separated the patients into two groups based on 25 (OH) vitamin D_3 level (<32 ng/ml vs. ≥32 ng/ml), and used the logistic regression to examine whether the SNPs genotypes were significantly associated with the 25 (OH) vitamin D_3 group status. Multiple testing corrections were controlled by the approach of Benjamin and Hochberg [17].

RESULTS

25(OH) Vitamin D_3 Levels in AML

There were 34 (35%) patients with normal 25(OH) vitamin D_3 level, 34 (35%) had insufficient levels and 29 (30%) had deficient levels. Similar distribution was found among a cohort of 100 healthy volunteers from Western New York [29 (29%) normal, 40 (40%) insufficient and 31 (31%) deficient [18].

Associations of 25(OH) Vitamin D_3 Levels with Pretreatment Clinical and Molecular Characteristics

No differences in age or gender were observed between patients with normal or low 25(OH) vitamin D_3 levels; not surprisingly, nonwhite patients (n=7) tended to have lower 25(OH) vitamin D_3 levels (P=0.02). Similarly, there were no differences in any of the other pretreatment clinical characteristics as outlined in Table 1 except that *FLT3* internal tandem duplication (ITD) was rarely present in patients with normal 25(OH) vitamin D_3 levels (P=0.02).

Associations of 25(OH) Vitamin D_3 Levels with Clinical Outcome

25(OH) Vitamin D_3 levels were not associated with the probability of CR attainment (Table 2). With a median follow up of 15.6 (range, 0.1 to 84.3) months for patients alive, those with insufficient and deficient 25(OH) vitamin D_3 levels compared to those with normal 25(OH) vitamin D_3 levels had significantly shorter RFS (P=0.025) by Kaplan Meier analysis; median RFS survival and 95%CI were 8.7 (5.9, 64.1) months, 5 (2.9, 12.1) months and 16.3 (10.5, 41.3) months, respectively (Figure 1A). Using the Cox hazard ratio analysis, the statistical difference was detected only between those with deficient levels and those with normal levels (Table 2). There was no significant difference in OS between those with insufficient and deficient 25(OH) vitamin D_3 levels as compared to those with normal 25(OH) vitamin D_3 levels using Kaplan Meier analysis; median OS survival and 95% CI were 12.5 (6.7, 64.1) months, 9.8 (2.9, 19.3) months and 25.2 (14.3, not reached) months, respectively (Figure 1B). Using the Cox hazard ratio analysis, patients with deficient 25(OH) vitamin D_3 levels had significantly higher hazard in OS compared to those with normal 25(OH) vitamin D_3 levels (Table 2).

The univariate analysis is presented in Table 3. In a multivariate model for RFS and OS (Table 4), 25(OH) vitamin D_3 level remained associated with outcome when adjusted for white blood cell count, smoking, age and European LeukemiaNetwork (ELN) Genetic Groups.

Association of 25(OH) Vitamin D_3 Levels with MiR-Expression Profile

To understand the biology of the effect of 25(OH) vitamin D_3 on AML outcome we analyzed miR expression. As mentioned in the Methods, the initial screen with whole genome microarray, using an unadjusted $P<0.05$ and at least two-fold expression level change, revealed that 13 miRs were up-regulated and four were down-regulated in patients with subnormal (<32 ng/ml) 25(OH) vitamin D_3 levels. No significant results were found after multiple test corrections. Of interest, miR144 was also up-regulated in samples from prostate cancer patients with subnormal 25(OH) vitamin D_3 level (Campbell M, unpublished data). We then analyzed 16 of these miRs by RT-PCR in an additional cohort of 58 patients on whom samples were available (44 with subnormal and 14 with normal 25(OH) vitamin D_3 levels). However, none of the specific signature miRs remained significantly associated with serum 25(OH) vitamin D_3 level (data not shown).

Association between 25(OH) vitamin D_3 levels and SNPs in the vitamin D pathway

To evaluate the possible contribution of pharmacogenetics to variations in serum 25(OH) vitamin D_3 status in AML patients, 90 genotyped tagSNPs in the genes encoding the vitamin D pathway enzymes (*CYP27A1*, *CYP2R1*, *CYP27B1*, *CYP24A1*, *GC* and *VDR*) were successfully analyzed. Using unadjusted P value <0.05, for the first analysis where the patients' 25 (OH) vitamin D_3 level was treated as continuous variable, there were six SNPs whose genotypes were significantly associated with the patients' 25(OH) vitamin D_3 level; for the second analysis where the patients' 25(OH) vitamin D_3 level was separated as two

groups (i.e., 25(OH) vitamin D_3<32 ng/ml vs. ≥32 ng/ml), there were six SNPs whose genotypes were significantly associated with 25(OH) vitamin D_3 levels (i.e., 25(OH) vitamin D_3<32 ng/ml vs. ≥32 ng/ml). A total of three SNPs were significant after both linear and logistic model analyses, namely *GC* SNPs rs4588 and rs2762934 and *VDR* SNP rs10783219. However, after adjusting for multiple comparisons, none were significant.

Association between VDR SNP and outcome

The six SNPs whose genotypes were significantly associated with the patients' 25(OH) vitamin D_3 levels were analyzed for correlation with outcome. In the *VDR* SNP, rs10783219, the presence of the T allele was significantly associated with inferior CR rate (P=0.0442), shorter RFS (P=0.0058) and OS (P=0.0011) (Figures 2A, B). In multivariate analysis, this SNP retained statistical significance for RFS and OS (Table 5A, B). Interestingly, there was a significant association between 25(OH) Vitamin D_3 level and rs10783219 genotype (P=0.0132). The 25(OH) Vitamin D_3 deficient group had more TA (62.5%) and the 25(OH) Vitamin D_3 normal group had more AA (69.6%). This may explain why 25(OH) Vitamin D_3 was not significant in the multivariate model when adjusted for rs10783219.

DISCUSSION

This is the first report to associate low 25(OH) vitamin D_3 levels with worse outcome in AML. This finding is in line with previous work in NHL [4] and CLL [5] showing that low 25(OH) vitamin D_3 levels were associated with worse outcome.

We are also the first to report an association between AML molecular subgroups and 25(OH) vitamin D_3 levels. The finding that normal 25(OH) vitamin D_3 levels are rarely associated with *FLT3*-ITD is intriguing. It is of further interest when one considers the miR data. We have previously shown that miR144 was up-regulated in AML with *FLT3*-ITD [19] and the fact that miR144 was also up-regulated in AML with low vitamin D raises questions about its role in *FLT3*-ITD leukemogenesis. In that regard, Gocek et al [20] attempted to differentiate AML cells with 1,25-dihydroxyvitamin D_3 and showed that blasts from AML with *FLT3*-ITD failed to differentiate in spite of elevated *VDR* expression suggesting that the failure lies downstream of the receptor.

On the other hand, AML with monosomy 7 or partial loss of 7q seemed extremely sensitive to 1,25-dihydroxyvitamin D_3 and demonstrated significant differentiation [20]. Considering that AML with monosomy 7 is a subset with especially poor outcome to conventional chemotherapy, vitamin D may prove a potential treatment adjunct in AML with this abnormality. In addition, even though our data did not support a correlation between vitamin D levels and FAB, others[21] have shown a correlation between functional VDR and FAB subtypes.

One shortcoming of this study is the relatively small sample size, which does not provide us enough power for some of the molecular markers (e.g., *NPM1*), miR or SNP analysis. Therefore, our small-size study is exploratory in nature and the data should be interpreted with caution. This is of importance as others[22] have shown that Vitamin D activated miR26a and thereby induced an anti-leukemic effect. Nevertheless, the observation that several SNPs examined here are associated with low 25(OH) vitamin D_3 levels and that one was associated with outcome is novel but raises some concerns, if confirmed by others. Specifically, if indeed a SNP signature is inherent in AML, supplementing these patients with vitamin D might not affect their outcome. It may, however, affect the microenvironment nurturing those leukemia cells. Further studies with larger sample size and functional experiments for understanding this mechanism are warranted.

Therefore, measuring 25(OH) vitamin D_3 levels may predict outcome in AML and studies to supplement with vitamin D compounds are warranted. The strengths of our study include the intensively-treated cohort of newly-diagnosed well characterized AML patients and the limitation is the small number of cases. The other limitation of our study is that we do not provide a causal relationship between low serum 25(OH) vitamin D_3 levels and worse outcome in AML. We have initiated a pharmacokinetic study of vitamin D supplementation and expect those results in one to two years.

In conclusion, our data provide the first evidence that serum 25(OH) vitamin D_3 levels may be an important factor influencing AML outcome, and that patients with *FLT3*-ITD rarely have normal vitamin D levels. It therefore seems that serum 25(OH) vitamin D_3 level is a modifiable and economically feasible patient risk factor, and raises the hypothesis that when judiciously intervened could possibly improve AML patient outcome without significant toxicity or cost.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported partially by grants from the National Cancer Institute Grant CA16056 (HJL, JM, WT, QH, DW, SL, GEW, LAF, SNJS, AWB, AAA, MB, EAG, JET, ESW, CSJ, DLT, MW), the Szefel Foundation, Roswell Park Cancer Institute (ESW), the Leonard S. LuVullo Endowment for Leukemia Research (MW), the Nancy C. Cully Endowment for Leukemia Research (MW), the Babcock Family Endowment (MW) and the Heidi Leukemia Research Fund, Buffalo, NY (MW).

REFERENCES

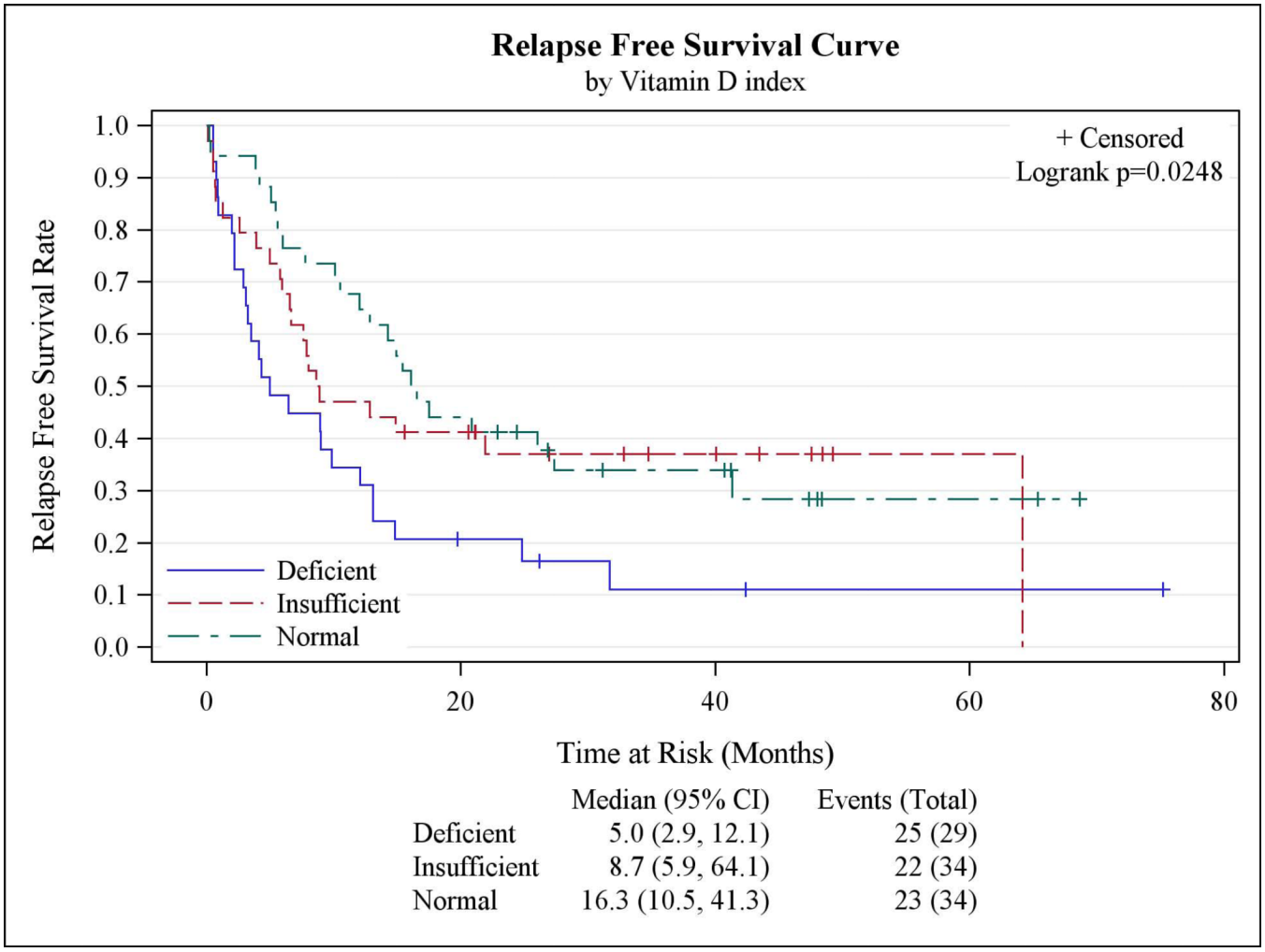
1. Hassan IB, Islam SI, Alizadeh H, Kristensen J, Kambal A, Sonday S, Bernseen RM. Acute leukemia among the adult population of United Arab Emirates: an epidemiological study. Leukemia & lymphoma. 2009; 50(7):1138–1147. [PubMed: 19557635]
2. Al-Turki HA, Sadat-Ali M, Al-Elq AH, Al-Mulhim FA, Al-Ali AK. 25-Hydoxyvitamin D levels among healthy Saudi Arabian women. Saudi medical journal. 2008; 29(12):1765–1768. [PubMed: 19082230]
3. Miyaura C, Abe E, Kuribayashi T, Tanaka H, Konno K, Nishii Y, Suda T. 1 alpha,25-Dihydroxyvitamin D3 induces differentiation of human myeloid leukemia cells. Biochemical and biophysical research communications. 1981; 102(3):937–943. [PubMed: 6946774]
4. Drake MT, Maurer MJ, Link BK, Habermann TM, Ansell SM, Micallef IN, Kelly JL, Macon WR, Nowakowski GS, Inwards DJ, Johnston PB, Singh RJ, Allmer C, Slager SL, Weiner GJ, Witzig TE, Cerhan JR. Vitamin D insufficiency and prognosis in non-Hodgkin's lymphoma. J Clin Oncol. 2010; 28(27):4191–4198. [PubMed: 20713849]
5. Shanafelt TD, Drake MT, Maurer MJ, Allmer C, Rabe KG, Slager SL, Weiner GJ, Call TG, Link BK, Zent CS, Kay NE, Hanson CA, Witzig TE, Cerhan JR. Vitamin D insufficiency and prognosis in chronic lymphocytic leukemia. Blood. 2011; 117(5):1492–1498. [PubMed: 21048153]
6. Shimizu T, Taira N, Senou M, Takeda K. Involvement of diverse protein kinase C isoforms in the differentiation of ML-1 human myeloblastic leukemia cells induced by the vitamin D3 analogue KH1060 and the phorbol ester TPA. Cancer letters. 2002; 186(1):67–74. [PubMed: 12183077]
7. (a) Wang X, Studzinski GP. Activation of extracellular signal-regulated kinases (ERKs) defines the first phase of 1,25-dihydroxyvitamin D3-induced differentiation of HL60 cells. Journal of cellular biochemistry. 2001; 80(4):471–482. [PubMed: 11169731] (b) Ji Y, Kutner A, Verstuyf A, Verlinden L, Studzinski GP. Derivatives of vitamins D2 and D3 activate three MAPK pathways and upregulate pRb expression in differentiating HL60 cells. Cell Cycle. 2002; 1(6):410–415. [PubMed: 12548017] (c) Hughes PJ, Brown G. 1Alpha,25-dihydroxyvitamin D3-mediated stimulation of steroid sulphatase activity in myeloid leukaemic cell lines requires VDRnuc-mediated activation of

the RAS/RAF/ERK-MAP kinase signalling pathway. Journal of cellular biochemistry. 2006; 98(3): 590–617. [PubMed: 16440327] (d) Marcinkowska E, Garay E, Gocek E, Chrobak A, Wang X, Studzinski GP. Regulation of C/EBPbeta isoforms by MAPK pathways in HL60 cells induced to differentiate by 1,25-dihydroxyvitamin D3. Experimental cell research. 2006; 312(11):2054–2065. [PubMed: 16624284] (e) Studzinski GP, Garay E, Patel R, Zhang J, Wang X. Vitamin D receptor signaling of monocytic differentiation in human leukemia cells: role of MAPK pathways in transcription factor activation. Current topics in medicinal chemistry. 2006; 6(12):1267–1271. [PubMed: 16848740]

8. Rovira C, Guida MC, Cayota A. MicroRNAs and other small silencing RNAs in cancer. IUBMB Life. 2010
9. (a) Orton SM, Morris AP, Herrera BM, Ramagopalan SV, Lincoln MR, Chao MJ, Vieth R, Sadovnick AD, Ebers GC. Evidence for genetic regulation of vitamin D status in twins with multiple sclerosis. The American journal of clinical nutrition. 2008; 88(2):441–447. [PubMed: 18689381] (b) Hunter D, De Lange M, Snieder H, MacGregor AJ, Swaminathan R, Thakker RV, Spector TD. Genetic contribution to bone metabolism, calcium excretion, and vitamin D and parathyroid hormone regulation. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2001; 16(2):371–378. [PubMed: 11204437]
10. Touvier M, Chan DS, Lau R, Aune D, Vieira R, Greenwood DC, Kampman E, Riboli E, Hercberg S, Norat T. Meta-analyses of vitamin d intake, 25-hydroxyvitamin d status, vitamin d receptor polymorphisms, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 2011; 20(5): 1003–1016. [PubMed: 21378269]
11. Chadha MK, Fakih M, Muindi J, Tian L, Mashtare T, Johnson CS, Trump D. Effect of 25-hydroxyvitamin D status on serological response to influenza vaccine in prostate cancer patients. The Prostate. 2011; 71(4):368–372. [PubMed: 20812224]
12. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD, Napoli JL. Determination of vitamin D status by radioimmunoassay with an 125I-labeled tracer. Clinical chemistry. 1993; 39(3):529–533. [PubMed: 8448871]
13. Rosen CJ. Clinical practice. Vitamin D insufficiency. The New England journal of medicine. 2011; 364(3):248–254. [PubMed: 21247315]
14. (a) Adjei AA, Thomae BA, Prondzinski JL, Eckloff BW, Wieben ED, Weinshilboum RM. Human estrogen sulfotransferase (SULT1E1) pharmacogenomics: gene resequencing and functional genomics. British journal of pharmacology. 2003; 139(8):1373–1382. [PubMed: 12922923] (b) Ma CX, Adjei AA, Salavaggione OE, Coronel J, Pelleymounter L, Wang L, Eckloff BW, Schaid D, Wieben ED, Weinshilboum RM. Human aromatase: gene resequencing and functional genomics. Cancer research. 2005; 65(23):11071–11082. [PubMed: 16322257] (c) Leil TA, Endo C, Adjei AA, Dy GK, Salavaggione OE, Reid JR, Ames MM. Identification and characterization of genetic variation in the folylpolyglutamate synthase gene. Cancer research. 2007; 67(18):8772–8782. [PubMed: 17875718]
15. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–265. [PubMed: 15297300]
16. Cheson BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, Schiffer CA, Doehner H, Tallman MS, Lister TA, Lo-Coco F, Willemze R, Biondi A, Hiddemann W, Larson RA, Lowenberg B, Sanz MA, Head DR, Ohno R, Bloomfield CD. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003; 21(24):4642–4649. [PubMed: 14673054]
17. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. 1995; 57(1):289–300.
18. Trump DL, Chadha MK, Sunga AY, Fakih MG, Ashraf U, Silliman CG, Hollis BW, Nesline MK, Tian L, Tan W, Johnson CS. Vitamin D deficiency and insufficiency among patients with prostate cancer. BJU international. 2009; 104(7):909–914. [PubMed: 19426195]
19. Whitman SP, Maharry K, Radmacher MD, Becker H, Mrozek K, Margeson D, Holland KB, Wu YZ, Schwind S, Metzeler KH, Wen J, Baer MR, Powell BL, Carter TH, Kolitz JE, Wetzler M, Moore JO, Stone RM, Carroll AJ, Larson RA, Caligiuri MA, Marcucci G, Bloomfield CD. FLT3 internal tandem duplication associates with adverse outcome and gene- and microRNA-expression

signatures in patients 60 years of age or older with primary cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. Blood. 2010; 116(18):3622–3626. [PubMed: 20656931]

20. Gocek E, Kielbinski M, Baurska H, Haus O, Kutner A, Marcinkowska E. Different susceptibilities to 1,25-dihydroxyvitamin D3-induced differentiation of AML cells carrying various mutations. Leukemia research. 2010; 34(5):649–657. [PubMed: 19880182]
21. Grande A, Manfredini R, Pizzanelli M, Tagliafico E, Balestri R, Trevisan F, Barbieri D, Franceschi C, Battini R, Ferrari S. Presence of a functional vitamin D receptor does not correlate with vitamin D3 phenotypic effects in myeloid differentiation. Cell death and differentiation. 1997; 4(6):497–505. [PubMed: 16465271]
22. Salvatori B, Iosue I, Mangiavacchi A, Loddo G, Padula F, Chiaretti S, Peragine N, Bozzoni I, Fazi F, Fatica A. The microRNA-26a target E2F7 sustains cell proliferation and inhibits monocytic differentiation of acute myeloid leukemia cells. Cell death & disease. 2012; 3:e413. [PubMed: 23096114]
23. Lee HJ, Licht AS, Hyland AJ, Ford LA, Sait SN, Block AW, Barcos M, Baer MR, Wang ES, Wetzler M. Is obesity a prognostic factor for acute myeloid leukemia outcome? Ann Hematol. 2012; 91(3):359–365. [PubMed: 21935651]



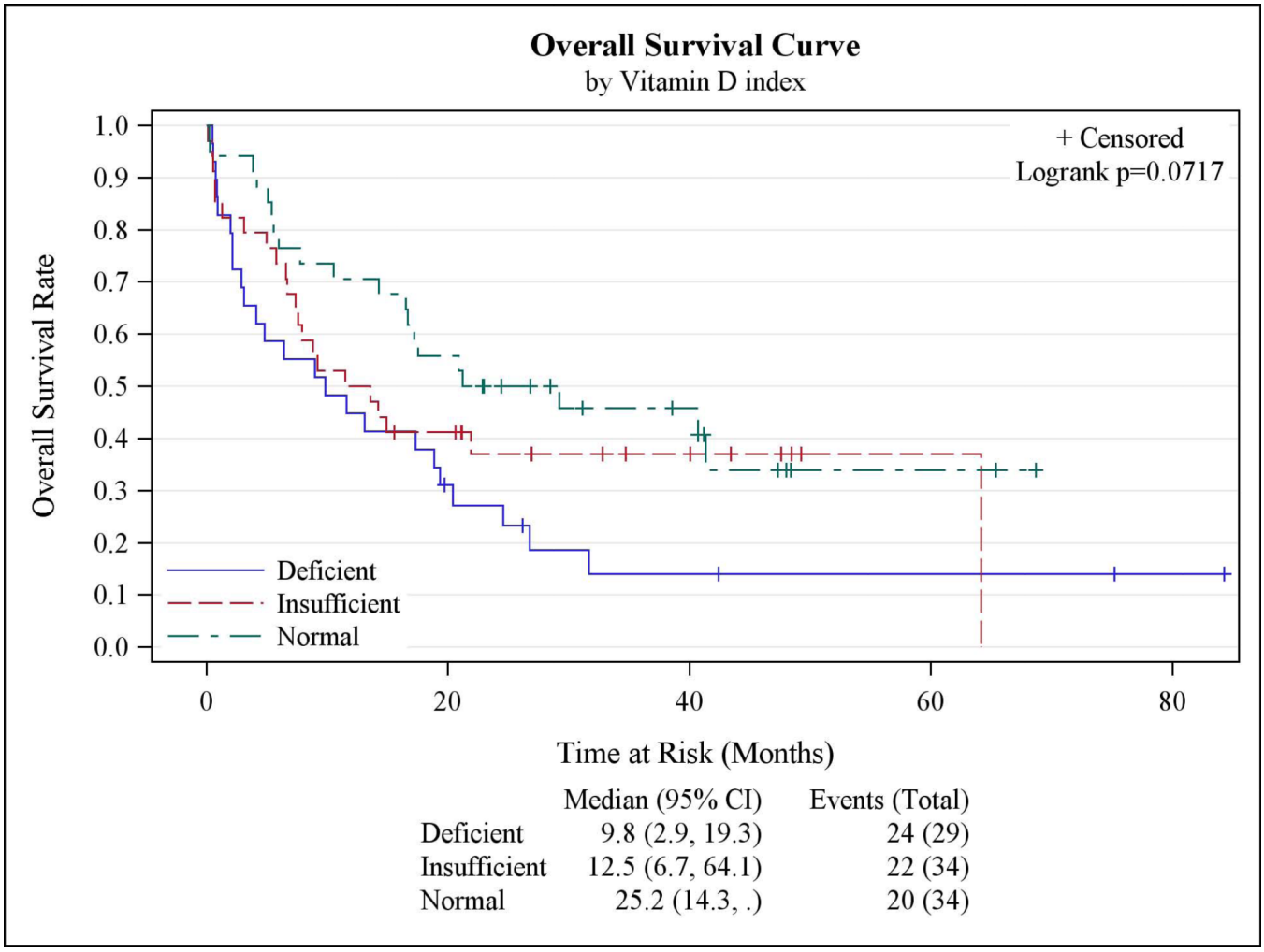
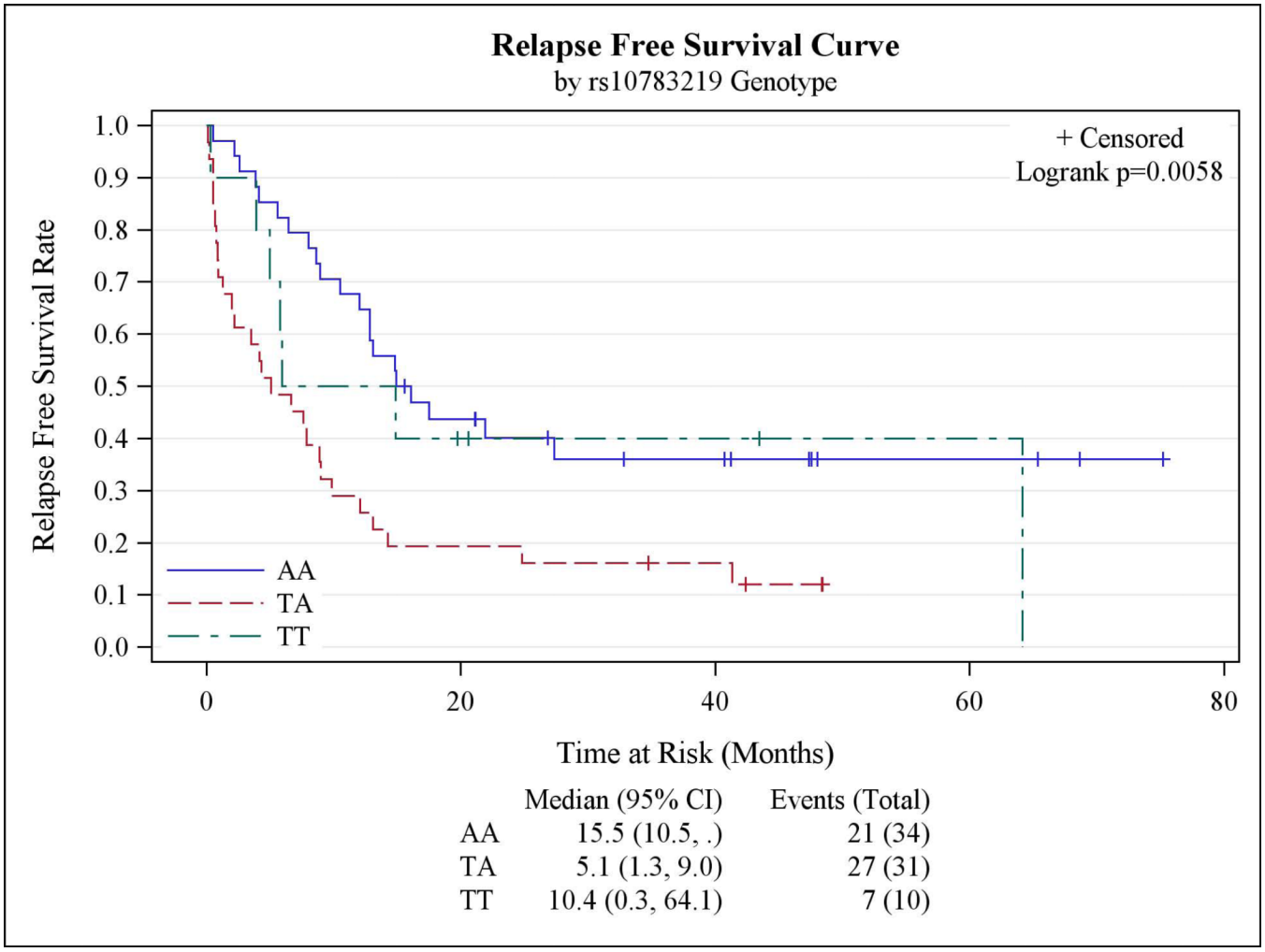


Figure 1.
Kaplan-Meier survival curves according to vitamin D levels. (A) Relapse-free survival and (B) overall survival.



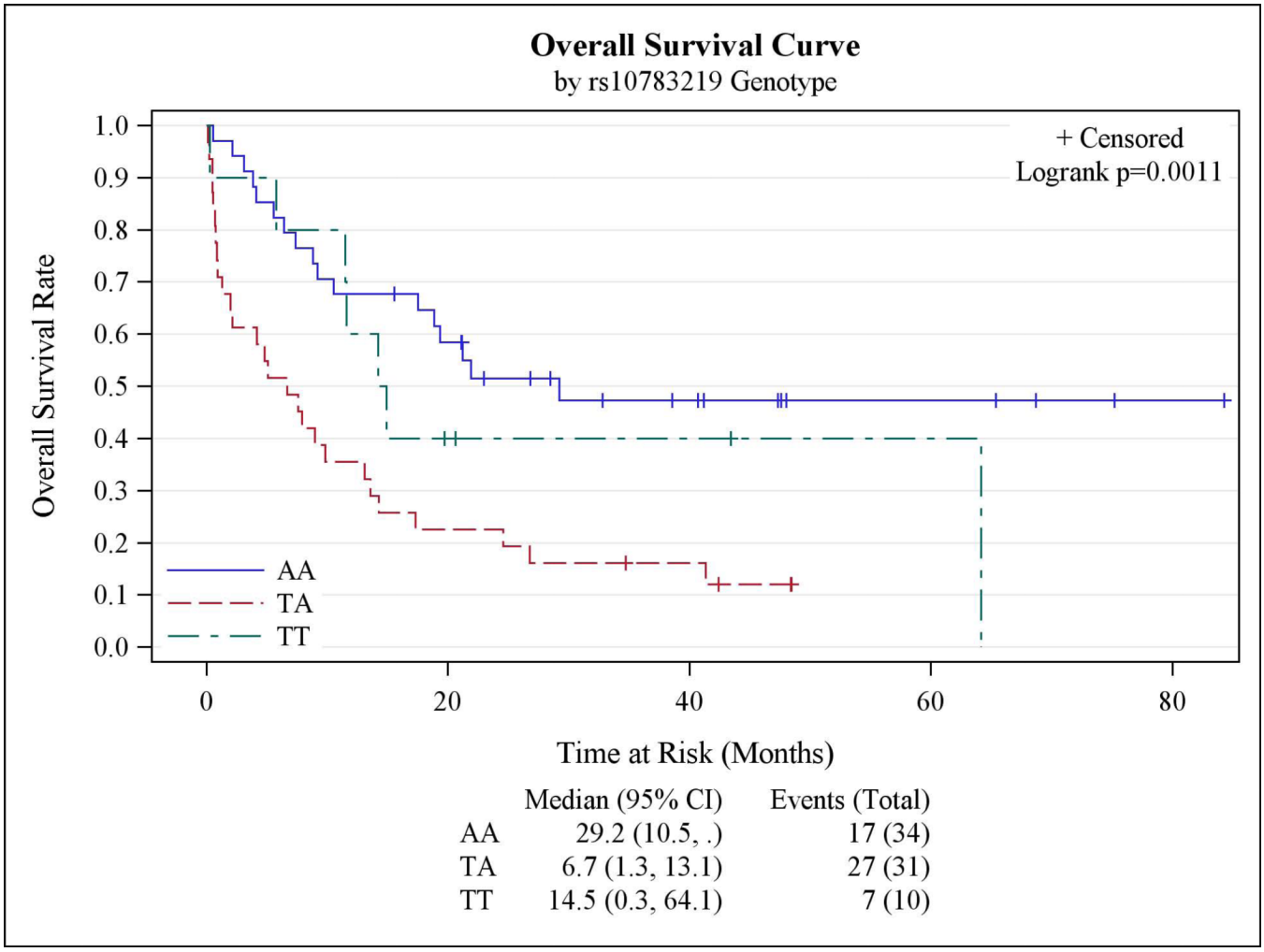


Figure 2.
Kaplan-Meier survival curves according to VDR SNP rs10783219. (A) Relapse-free survival and (B) overall survival.

Table 1

Patient characteristics according to vitamin D level

Patient characteristics	Deficient (<20 ng/ml) N=29 (30%)	Insufficient (20–31.9 ng/ml) N=34 (35%)	Normal (32–100 ng/ml) N=34 (35%)	*P*
Age (years)				0.71
<60	16 (55.2%)	15 (44.1%)	16 (47.1%)	
≥60	13 (44.8%)	19 (55.9%)	18 (52.9%)	
Gender				0.28
Male	12 (41.4%)	21 (61.8%)	17 (50%)	
Female	17 (58.6%)	13 (38.2%)	17 (50%)	
Race				**0.03**
White	23 (82.1%)	32 (94.1%)	34 (100%)	
Nonwhite	5 (17.9%)	2 (5.9%)	0 (0%)	
WBC count $\times 10^9$/L				0.32
Median	21.8	5.4	15.7	
Range	(1.1, 555.2)	(0.7, 292.6)	(0.6, 186.6)	
Percentage of PB blasts				0.82
Median	25	35	36.5	
Range	(0, 96)	(0, 92)	(0, 92)	
Percentage of BM blasts				0.94
Median	58	68	57.5	
Range	(21, 92)	(18, 95)	(20, 97)	
FAB category				0.15
M0	2 (7.1%)	1 (3.1%)	2 (6.7%)	
M1	7 (25%)	9 (28.1%)	9 (30%)	
M2	10 (35.7%)	6 (18.8%)	12 (40%)	
M4	8 (28.6%)	8 (25%)	2 (6.7%)	
M5	1 (3.6%)	7 (21.9%)	4 (13.3%)	
M6	0 (0%)	0 (0%)	1 (3.3%)	
M7	0 (0%)	1 (3.1%)	0 (0%)	
BMI[23]				0.53
Underweight	1 (3.4%)	1 (2.9%)	2 (5.9%)	
Normal	10 (34.5%)	7 (20.6%)	11 (32.4%)	
Overweight	6 (20.7%)	16 (47.1%)	12 (35.3%)	
Obese	5 (17.2%)	6 (17.6%)	5 (14.7%)	
Very obese	7 (24.1%)	4 (11.8%)	4 (11.8%)	
Smoking				0.17

Patient characteristics	**Deficient (<20 ng/ml) N=29 (30%)**	**Insufficient (20–31.9 ng/ml) N=34 (35%)**	**Normal (32–100 ng/ml) N=34 (35%)**	***P***
Current Smoker	10 (34.5%)	10 (29.4%)	4 (11.8%)	
Previous Smoker	10 (34.5%)	11 (32.4%)	11 (32.4%)	
Nonsmoker	9 (31%)	13 (38.2%)	19 (55.9%)	
AML Presentation				0.47
De novo	19 (65.5%)	27 (79.4%)	26 (76.5%)	
Secondary*	10 (34.5%)	7 (20.6%)	8 (23.5%)	
NPM1				0.16
Mutated	8 (27.6%)	8 (25%)	3 (9.4%)	
Wild type	21 (72.4%)	24 (75%)	29 (90.6%)	
FLT3-ITD				**0.02**
Present	7 (24.1%)	8 (23.5%)	1 (3%)	
Absent	22 (75.9%)	26 (76.5%)	32 (97%)	
ELN Genetic Group				0.45
Favorable	4 (16%)	11 (34.4%)	7 (21.9%)	
Intermediate-I	11 (44%)	10 (31.3%)	11 (34.4%)	
Intermediate-II	4 (16%)	8 (25%)	6 (18.8%)	
Adverse	6 (24%)	3 (9.4%)	8 (25%)	

*Secondary includes both presence of antecedent hematologic disorder and therapy-related

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; BMI, body mass index; ELN, European LeukemiaNet; FAB, French-American-British; PB, peripheral blood; WBC, white blood cell;

Table 2

Patient Outcome according to vitamin D levels

End point	Deficient (<20 ng/ml) N=29 (30%)	Insufficient (20–31.9 ng/ml) N=34 (35%)	Normal (32–100 ng/ml) N=34 (35%)	P (Deficient vs. Normal)	P (Insufficient vs. Normal)
Complete remission					
Odds ratio	1.636	1.267	1	0.3379	0.6272
95% CI	(0.598, 4.48)	(0.488, 3.289)			
Relapse-free survival					
Hazard ratio	2.094	1.185	1	**0.0112**	0.5707
95% CI	(1.183, 3.707)	(0.66, 2.127)			
Overall survival					
Hazard ratio	1.987	1.395	1	**0.0241**	0.2822
95% CI	(1.094, 3.607)	(0.76, 2.56)			

Abbreviations: CI, confidence interval;

Table 3

Univariate Survival Analysis

Group	Relapse-free survival		Overall survival	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
25(OH) Vitamin D_3 level				
Insufficient vs. Normal	1.185 (0.66, 2.127)	0.5707	1.395 (0.76, 2.56)	0.2822
Deficient vs. Normal	2.094 (1.183, 3.707)	**0.0112**	1.987 (1.094, 3.607)	**0.0241**
White blood cell count				
$<100 \times 10^9$/L vs. $\geq 100 \times 10^9$/L	0.164 (0.081, 0.331)	**<.0001**	0.294 (0.148, 0.586)	**0.0005**
Smoking status				
Current Smoker vs. Nonsmoker	0.643 (0.341, 1.215)	0.1736	0.623 (0.322, 1.206)	0.1604
Previous Smoker vs. Nonsmoker	1.057 (0.625, 1.789)	0.8364	1.099 (0.64, 1.886)	0.732
Age				
Each 10 year increase*	1.174 (1.016, 1.356)	**0.0299**	1.255 (1.076, 1.464)	**0.0039**
ELN Genetic Groups				
Intermeidate-1 vs. Favorable	1.398 (0.728, 2.685)	0.3137	1.422 (0.715, 2.826)	0.3155
Intermediate-II vs. Favorable	0.802 (0.351, 1.835)	0.6019	0.993 (0.424, 2.324)	0.9875
Adverse vs. Favorable	2.484 (1.204, 5.126)	**0.0138**	2.788 (1.297, 5.991)	**0.0086**

*Age was fit at 10-year increments to model a difference between every 10 years as opposed to annual increments.

Abbreviations: CI, confidence interval; ELN, European LeukemiaNetwork;

Table 4

Multivariate Survival Analysis

Group	Relapse-free survival		Overall survival	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Vitamin D level				
Insufficient vs. Normal	1.645 (0.819, 3.307)	0.162	2.227 (1.067, 4.65)	**0.033**
Deficient vs. Normal	2.588 (1.283, 5.22)	**0.0079**	2.927 (1.388, 6.171)	**0.0048**
White blood cell count				
$<100 \times 10^9$/L vs. $\geq 100 \times 10^9$/L	0.105 (0.044, 0.247)	**<.0001**	0.166 (0.071, 0.386)	**<.0001**
Smoking status				
Current Smoker vs. Nonsmoker	0.315 (0.147, 0.677)	**0.0031**	0.303 (0.135, 0.68)	**0.0038**
Previous Smoker vs. Nonsmoker	0.754 (0.414, 1.373)	0.3553	0.746 (0.405, 1.374)	0.3477
Age				
Each 10 year increase	1.154 (0.99, 1.347)	0.0679	1.273 (1.082, 1.497)	**0.0036**
ELN Genetic Groups				
Intermeidate-1 vs. Favorable	1.171 (0.585, 2.346)	0.656	1.209 (0.586, 2.494)	0.6071
Intermediate-II vs. Favorable	0.893 (0.378, 2.109)	0.797	1.199 (0.499, 2.883)	0.6852
Adverse vs. Favorable	3.179 (1.428, 7.079)	**0.0046**	3.997 (1.707, 9.364)	**0.0014**

Abbreviations: ELN, European LeukemiaNet;

Table 5

A: Multivariate analyses; effect of markers on clinical outcome (by Type 3 tests)

	P Value		
Effect	**CR**	**RFS**	**OS**
rs10783219	0.1458	**0.0183**	**0.0092**
Vitamin D level	0.0920	0.5076	0.3336
White blood cell count	**0.0059**	**<0.0001**	**<0.0001**
Smoking	0.8191	**0.0009**	**0.0021**
Age	0.2310	0.1676	**0.0197**
ELN Genetic Groups	**0.0182**	**0.0137**	**0.0057**

B: Multivariate analyses; effect of markers on clinical outcome with Odds/Hazard ratios and 95% confidence intervals (CIs)

Group	**Complete remission**		**Relapse-free survival**		**Overall survival**	
	Odds ratio (95% CI)	**P**	**Hazard ratio (95% CI)**	**P**	**Hazard ratio (95% CI)**	**P**
rs10783219						
TA vs. AA	0.344 (0.066, 1.793)	0.2051	2.55 (1.199, 5.422)	**0.0150**	3.113 (1.385, 6.994)	**0.0060**
TT vs. AA	3.907 (0.224, 68.148)	0.3501	0.936 (0.297, 2.95)	0.9094	1.11 (0.349, 3.532)	0.8596
Vitamin D level						
Insufficient vs. Normal	1.896 (0.336, 10.699)	0.4688	1.558 (0.688, 3.531)	0.2876	1.945 (0.807, 4.689)	0.1386
Deficient vs. Normal	10.62 (1.202, 93.8)	**0.0335**	1.574 (0.642, 3.862)	0.3217	1.546 (0.599, 3.987)	0.3674
White blood cell count						
$<100\times10^9$/L vs. $\geq100\times10^9$/L	25.161 (2.531, 250.09)	**0.0059**	0.087 (0.034, 0.223)	**<.0001**	0.133 (0.052, 0.336)	**<.0001**
Smoking status						
Current Smoker vs. Nonsmoker	1.571 (0.268, 9.205)	0.6168	0.171 (0.068, 0.432)	**0.0002**	0.187 (0.073, 0.476)	**0.0004**
Previous Smoker vs. Nonsmoker	1.619 (0.289, 9.064)	0.5835	0.495 (0.235, 1.042)	0.0642	0.548 (0.258, 1.167)	0.1188
Age						
Each 10 year increase	0.788 (0.534, 1.164)	0.2310	1.142 (0.946, 1.38)	0.1676	1.265 (1.038, 1.541)	**0.0197**
ELN Genetic Groups						
Intermeidate-1 vs. Favorable	0.05 (0.005, 0.457)	**0.0080**	0.9 (0.404, 2.003)	0.7959	1.191 (0.518, 2.738)	0.6815

B: Multivariate analyses; effect of markers on clinical outcome with Odds/Hazard ratios and 95% confidence intervals (CIs)

Group	Complete remission		Relapse-free survival		Overall survival	
	Odds ratio (95% CI)	P	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Intermediate-II vs. Favorable	0.072 (0.006, 0.801)	**0.0324**	0.827 (0.314, 2.183)	0.7018	1.235 (0.457, 3.339)	0.6777
Adverse vs. Favorable	0.013 (0.001, 0.202)	**0.0020**	4.556 (1.593, 13.025)	**0.0047**	6.548 (2.19, 19.579)	**0.0008**

Abbreviations: CR, complete remission; ELN, European LeukemiaNet; OS, overall survival; RFS, relapse-free survival; SNP, single nucleotide polymorphism;

Abbreviations: ELN, European LeukemiaNet;