Variability in responsiveness to lovastatin of the primitive CD34 AML subfraction compared to normal CD34 cells

Susan D. P. W. M. Jonge-Peeters, Karen Weide, Folkert Kuipers, Wim J. Sluiter, Elisabeth G. E. Vries, Edo Vellenga

► To cite this version:

Susan D. P. W. M. Jonge-Peeters, Karen Weide, Folkert Kuipers, Wim J. Sluiter, Elisabeth G. E. Vries, et al.. Variability in responsiveness to lovastatin of the primitive CD34 AML subfraction compared to normal CD34 cells. *Annals of Hematology*, 2008, 88 (6), pp.573-580. 10.1007/s00277-008-0633-2 . hal-00477989

HAL Id: hal-00477989

<https://hal.science/hal-00477989>

Submitted on 30 Apr 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Variability in responsiveness to lovastatin of the primitive CD34⁺ AML subfraction compared to normal CD34⁺ cells

Susan D. P. W. M. de Jonge-Peeters ·
Karen van der Weide · Folkert Kuipers ·
Wim J. Sluiter · Elisabeth G. E. de Vries · Edo Vellenga

Received: 25 February 2008 / Accepted: 20 October 2008 / Published online: 11 November 2008
© Springer-Verlag 2008

Abstract In the present study, we questioned whether the cholesterol synthesis inhibitor lovastatin potentiates the cytotoxicity of chemotherapeutic agents in the primitive CD34⁺ subpopulation of acute myeloid leukemia (AML) cells. AML mononuclear cells ($n=17$) were sorted in CD34⁺ and CD34⁻ fractions and compared to normal CD34^{+/+} cells ($n=7$). The percentage of surviving cells upon exposure to lovastatin (25–100 μ M) and/or chemotherapeutics (cytarabine or daunorubicin) was determined with a luminescent cell viability assay. The results demonstrate that the primitive CD34⁺ subpopulation of normal and AML cells displayed a higher sensitivity to

lovastatin than the more mature CD34⁻ subpopulation. The combination of lovastatin and chemotherapeutics resulted in a more pronounced inhibitory effect on both subpopulations. In contrast to the homogeneous results in normal CD34⁺ cells, a distinct heterogeneity in lovastatin sensitivity was found in AML samples. Therefore, a group of normal ($n=11$) and abnormal ($n=6$) responders were identified based on a reduced or increased cell survival compared to normal CD34⁺ cells. This distinction was not only observed in the CD34⁺ AML subfraction but also in CD34⁺CD38⁻ AML cells. In the abnormal responder group, 50% of patients presented with unfavorable cytogenetics and significant higher peripheral blast cell counts, which coincided with poor treatment results. In summary, the findings indicate that the primitive subfraction of CD34⁺ AML cells is in the majority of cases affected by lovastatin treatment, which is potentiated when combined with chemotherapeutics. Heterogeneity of the response observed in AML patients allowed identification of a subgroup with poor prognosis.

Keywords AML · Cholesterol metabolism · Cytostatic agents · CD34 subpopulations · ABC transporters

S. D. P. W. M. de Jonge-Peeters · K. van der Weide · E. Vellenga
Department of Hematology, University of Groningen
and University Medical Center Groningen,
P.O. Box 30.001, 9700 RB Groningen, The Netherlands

S. D. P. W. M. de Jonge-Peeters · K. van der Weide ·
E. G. E. de Vries
Department of Medical Oncology, University of Groningen
and University Medical Center Groningen,
P.O. Box 30.001, 9700 RB Groningen, The Netherlands

F. Kuipers
Department of Pediatrics, University of Groningen and University
Medical Center Groningen,
P.O. Box 30.001, 9700 RB Groningen, The Netherlands

W. J. Sluiter
Department of Endocrinology, University of Groningen
and University Medical Center Groningen,
P.O. Box 30.001, 9700 RB Groningen, The Netherlands

E. Vellenga (✉)
Department of Hematology,
University Medical Center Groningen,
Hanzeplein 1,
9713 GZ Groningen, The Netherlands
e-mail: e.vellenga@int.umcg.nl

Introduction

Acute myeloid leukemia (AML) is characterized by accumulation of immature myeloid cells in the bone marrow. These malignant cells are usually hierarchically structured, similar to the normal hematopoietic system. We have reported that, according to this hierarchical structure, ATP-binding cassette (ABC) transporters are expressed in a differentiation-dependent manner [1]. Specifically, in primitive CD34⁺CD38⁻ cells, a high expression of a number of ABC transporters genes was noticed in comparison to the

more differentiated CD34⁺CD38⁺ cells. Notably, in the leukemic counterpart, a much more heterogeneous pattern of ABC transporter gene expression was observed [1]. Recently, de Grouw et al. [2] published expression data for all 45 ABC transporters in AML cells. They found 22 ABC transporters to be lower expressed in the CD34⁺CD38⁺ subpopulation compared to the CD34⁺CD38[−] subpopulation. In addition to the two major cholesterol efflux transporters (ABCA1 and ABCG1), two genes critical in cholesterol metabolism (3-hydroxy-3-methyl-glutaryl-CoA (HMGCoA) reductase and low-density lipoprotein receptor (LDLR)) were highly expressed in normal CD34⁺CD38[−] cells, indicative for an active cholesterol metabolism [1, 3].

In AML cells, an aberrant cellular cholesterol metabolism has been demonstrated, including a higher LDLR activity [4]. Interestingly, it was recently shown in vitro that cells isolated from a subgroup of AML patients demonstrated in their leukemic cells “an acute cholesterol response”, i.e., a rapid increase in cellular cholesterol content in response to cytotoxic agents [5]. These AML cells were further characterized by an increased chemosensitivity upon blocking this acute cholesterol response by pretreatment of cells with a statin [4]. Statins act as competitive inhibitors of the enzyme HMGCoA reductase and hence block cellular cholesterol synthesis. Consequently, a role for these cholesterol synthesis inhibitors to improve standard antileukemic treatment has been suggested [4, 6–10]. In the present study, we focused on a subpopulation of AML cells (CD34⁺), i.e., cells that have more in common with the more primitive leukemic progenitor/stem cell compartment [11], and questioned whether this subpopulation of cells is especially prone for the effects of lovastatin and chemotherapeutic agents. Our data demonstrate a higher sensitivity of the primitive CD34⁺ subpopulation for lovastatin compared to the more mature CD34[−] subpopulation of normal as well as AML cells. Heterogeneity of the response observed in AML patients allowed identification of a subgroup with poor prognosis.

Materials and methods

Normal CD34^{+/−} hematopoietic cells

Normal mobilized peripheral blood cells were collected either from healthy donors or from patients awaiting autologous stem cell transplantation, undergoing granulocyte colony-stimulating factor (G-CSF) treatment, in accordance with institutional guidelines. CD34⁺, CD34[−]CD14⁺CD15⁺ (CD34[−]), CD34⁺CD38[−], and CD34⁺CD38⁺ cells were obtained by means of the MoFlo flow cytometer (Dako Colorado Inc., Fort Collins, CO, USA).

Patients

After informed consent, bone marrow or peripheral blood cells were collected of AML patients at diagnosis. The Human Subject Review Board of the University Medical Center Groningen (The Netherlands) approved the protocol. Patients were classified according to the WHO classification [12]. Mononuclear cells were enriched by Ficoll-Isopaque (Nycomed, Oslo, Norway) density gradient centrifugation and cryopreserved in RPMI 1640 medium (BioWhittaker, Brussels, Belgium) supplemented with 20% fetal calf serum (FCS; Hyclone, Logan, UT, USA) and 20% dimethyl sulfoxide (Merck, Amsterdam, The Netherlands) and stored at −196°C as described [13].

Materials

Mevinolin (L-154, 803-00G17) in the lactone form was obtained from Merck, Sharp & Dohme Research Laboratories (Rahway, NJ, USA), converted to its sodium salt, and a stock solution of mevinolin (lovastatin) at a concentration of 4 mg/mL was prepared as previously described. The cytotoxic agents daunorubicin (Aventis Pharma BV, Hoevelaken, The Netherlands) and cytarabin (Mayne Pharma (Benelux) SA-NV, Brussels, Belgium) were used.

Flow cytometric sorting

The normal and AML samples were incubated with a fluorescein isothiocyanate-conjugated antibody against CD34 (Becton Dickinson, San Jose, CA, USA) and a phycoerythrin-conjugated antibody against CD14 and CD15 or CD38. Sorting was performed using a MoFlo flow cytometer. Erythrocytes and dead cells were excluded from analysis by gating on forward and sideward light scatter. For RNA extraction, at least 3×10⁴ CD34⁺ and 3×10⁴ CD34[−]CD14⁺CD15⁺ cells were sorted. No difference existed in average CD34 expression between normal and leukemic cells mean fluorescence intensity.

Luminescent cell viability assay and TaqMan PCR

This assay was used for determining the number of viable cells in culture based on quantitation of the ATP present, which signals the presence of metabolically active cells. The amount of ATP is directly proportional to the number of cells present in the culture. The ATP measurement was carried out according to the manufacturer's instructions using the Cell Titer-Glo[®] Luminescent cell viability assay (Promega, Madison, WI, USA). We prepared 96-well plates with 100 μL RPMI 1640 medium supplemented with 10% FCS and sorted by the MoFlo flow cytometer, either 10⁴ CD34⁺ cells or CD34[−]CD14⁺CD15⁺ cells per well. The

wells were incubated with different concentrations of lovastatin (25, 50, and 100 μM) with and without different concentrations of either daunorubicin (0.01–0.5 μM) or cytarabine (0.0001–0.1 mg/mL) and analyzed after 20 h. All experiments were performed in duplicate. No difference existed in baseline average and median ATP levels between CD34^+ and CD34^- cells. RNA extraction, cDNA synthesis, and quantitative polymerase chain reaction were performed as described before [13].

Colony-forming cell assay

10^3 normal or AML CD34^+ cells were sorted into Iscove's Modified Dulbecco's Media (IMDM) containing 10% FCS, 20 ng/mL interleukin-3 (IL-3), 100 ng/mL c-Kit ligand, 100 ng/mL Flt-3 ligand, and 100 ng/mL thrombopoietin as described. Twenty-four hours after treatment with different concentrations lovastatin, cytarabine, and daunorubicin, cells were plated into MethoCult H4230 (StemCell Technologies, Vancouver, Canada) supplemented with 20% IMDM, 20 ng/mL IL-3, 20 ng/mL G-CSF, 20 ng/mL IL-6, 20 ng/mL cKit ligand, and 1 U/mL EPO (Cilag; Eprex, Brussels, Belgium).

Statistical analysis

Friedman's and Mann–Whitney's nonparametric tests were performed to calculate significant differences, p values of <0.05 . SPSS 14.0 statistical software [14] was used to analyze the data. Additionally, we looked for additive and synergistic effects of the combination treatment of lovastatin and chemotherapeutics. Additivity was defined as an increased inhibitory effect on cell survival of the combination exposure compared to the single exposure of lovastatin or chemotherapeutics. Synergism was defined by two individual agents (lovastatin and chemotherapeutics) acting together and creating an inhibitory effect on cell survival greater than predicted by knowing only the separate inhibitory effects of the individual agents.

Results

Patient characteristics

The AML patients ($n=17$) studied, median age of 51 (range 19–79), were classified according to the WHO classification: AML with cytogenetic abnormalities ($n=5$), AML with prior dysplastic syndrome ($n=2$), and the additional group consisting of M1 ($n=4$), M2 ($n=3$), and M4/M5 ($n=3$). The clinical and cellular characteristics of the patients are shown in Table 1. The median peripheral blast cell count at diagnosis was $48.6 \times 10^9/\text{L}$ (range 2.2–200). The majority

of the patients were treated according to ongoing Haemato-Oncology Co-operative Group (HOVON) protocols, i.e., the HOVON Swiss Group for Clinical Cancer Research AML-42 study for patients <60 years [15] and the HOVON 43 study for patients >60 years [16]. Palliative treatment was given to patients that were ineligible for intensive chemotherapy ($n=2$). These patients were treated with 6-mercaptopurine [17]. Eleven patients reached complete remission (CR) on protocol. In six patients, CR was not attained due to progression ($n=2$) or to treatment related toxicity ($n=4$). The median event-free survival (EFS) of all patients attaining CR was 13 months (range 1–37).

The CD34^+ subpopulation of normal and AML hematopoietic cells is more sensitive to lovastatin treatment than the more mature CD34^- subpopulation

To evaluate whether differences exist in statin sensitivity between primitive and more differentiated cells, normal CD34^+ and $\text{CD34}^-14^+15^+$ subfractions ($n=7$) were sorted from the mononuclear cell fraction from normal peripheral blood stem cells. A clear discrepancy between both fractions was observed upon lovastatin exposure. The CD34^+ subpopulation demonstrated a reduction of cellular ATP to 64% at a dose of 50 μM lovastatin compared to 123% in the CD34^- fraction ($p=0.008$; Fig. 1a).

A similar experimental setup was used for the AMLs ($n=17$). From the total AML cell fraction, the CD34^+ subfraction was separated from the CD34^- fraction by MoFlo. The percentage of CD34^+ cells varied strongly between the different AML samples as depicted in Table 1 (median 41% (range 7–92%)). Subsequently, the CD34^+ and CD34^- fractions were exposed to varying concentrations of lovastatin (25–100 μM) for 20 h and the effects on cell survival were assessed. As depicted in Fig. 1b, a significant difference in sensitivity for lovastatin was observed for the AML CD34^+ versus CD34^- fraction. At 50 μM , a median reduction of 30% in survival (range minus 24–54) was observed for the CD34^+ cells compared to 12% (range minus 51–42) in the CD34^- fraction ($p=0.029$). A similar pattern was also observed at 100 μM (43% versus 23%, $p=0.029$). Subsequently, we compared the AML subpopulations with the normal CD34^+ and CD34^- cell fractions and observed a significant increased lovastatin sensitivity of the AML CD34^- fraction compared to the normal the CD34^- subpopulations ($p=0.005$; Fig. 1). This was not noticed for the AML and normal CD34^+ cell fractions ($p=0.095$). Based on the pattern of lovastatin sensitivity of the normal CD34^+ cells, two subgroups within the AMLs could be distinguished. Eleven AML CD34^+ samples had a response pattern comparable to normal CD34^+ cells, whereas six showed a reduced lovastatin sensitivity (Fig. 3a; Table 1). A

Table 1 Clinical and cellular characteristics of AML patients

AMLs	Age (years)	Leucocytes presentation ($\times 10^9/L$)	FAB class	Cytogenetics	Treatment (it/pt)	Response	EFS ^a (months)	OS	% CD34 ⁺ in AML mononuclear cell fraction	(Ab)normal responder to lovastatin in CD34 ⁺ cells	Risk-group stratification ^b
1	59	128	M1	N	it	CR	18	1	24	N	Intermediate
2	68	4	M5b	N	it	CR	15	0	64	N	Intermediate
3	51	33	M1	N	it	CR	37	1	63	N	Intermediate
4	73	6	M2	N	it	CR	12	1	28	N	Intermediate
5	79	96	M5b	N	pt	NR	—	0	15	N	Intermediate
6	19	10	M2	N	it	CR	13	1	23	N	Intermediate
7	43	24	M5	N	it	CR	1	0	28	N	Intermediate
8	64	11	M5	inv. 16	it	CR	8	1	20	N	Good
9	48	16	M1	N	it	CR	8	0	17	N	Intermediate
10	49	50	M4/M5	N	it	CR	2.5	0	7	N	Intermediate
11	55	2	M2	N	it	CR	15	0	56	N	Intermediate
12	44	66	M5	3q-,5q-,+8	it	NE ^{tox}	0	0	8	A	Poor
13	54	48	M5a	N	it	NE ^{tox}	0	0	85	A	Intermediate
14	67	63	M1	T(6;9), Trisomy 13	it	NR	0	0	35	A	Poor
15	19	102	M5	inv. 16	it	CR	21+	1	60	A	Intermediate
16	42	200	M1	inv. 3q, -7, -10	it	NR	0	0	87	A	Poor
17	38	96	M1	N	pt	NR	—	0	92	A	Intermediate

N normal, *inv.* inversion, *it* intensive treatment, *pt* palliative treatment, *CR* complete remission, *NR* nonresponder, *NE* not evaluable due to treatment related toxicity, *EFS* event-free survival, *OS* overall survival, *0* death, *1* alive, *N* normal responders (defined as AML CD34⁺ cells demonstrating a similar effect on cell survival as normal CD34⁺ cells), *A* abnormal responders (defined as AML CD34⁺ showing a dissimilar effect on cell survival to normal CD34⁺ cells)

^aFor event-free survival, a plus following the value indicates still no relapse as at September 12, 2007

^bRisk group stratification is based on (un)favorable cytogenetics combined with peripheral blood blast cell counts

comparable pattern was observed for the AML CD34⁺ fraction (Fig. 3b).

The combination of lovastatin and chemotherapeutics potentiates cell death

To evaluate whether the cytotoxic effects of two frequently used cytostatic agents, cytarabine and daunorubicin, might be promoted by cotreatment with lovastatin, the AML CD34⁺ and CD34⁺ subfractions were incubated with varying concentrations of lovastatin (25, 50, and 100 μ M) in the presence or absence of varying concentrations of daunorubicin (0.01–0.5 μ M) or cytarabine (0.0001–0.1 mg/mL) for 24 h. A dose-dependent decrease in cell survival was seen as depicted in Fig. 2, which was augmented by lovastatin treatment. A median survival reduction of 21% (range minus 28–64%) was accomplished by exposing the AML CD34⁺ cells to 0.1 μ M daunorubicin, which was 43% (range minus 56–78%) when the CD34⁺ AML cells were exposed to the combination of daunorubicin and 25 μ M lovastatin (Fig. 2a).

The combined use was slightly more effective in the CD34⁺ subpopulation compared to the CD34⁺ subpopulation (Fig. 2b; median 43% versus 36%) but the difference

did not reach statistical significance. Comparable results were obtained for cytarabine (data not shown). However, also here within the group of AMLs, different subgroups could be distinguished when looking at cell survival. Most AML CD34⁺ cells with a reduced statin sensitivity demonstrated also a diminished susceptibility for the combined use of statins plus cytarabine or daunorubicin (Fig. 3). To investigate whether the observed effects were also noticed in the most primitive AML cell population, AML CD34⁺ cells were sorted into the CD34⁺CD38⁺ cell fraction and the CD34⁺CD38⁺ fraction ($n=7$). Four AML samples belonged to the normal responder group and three samples to the abnormal responder group. As depicted in Fig. 4, the same distinction in responsiveness to cytostatic agents was observed in the absence or presence of lovastatin. Especially in the CD34⁺CD38⁺ subfraction, the discrepancy between normal versus abnormal responders was most noticeable (e.g., for ARA-C in combination with 25 μ M lovastatin, $p=0.01$ for CD34⁺CD38⁺ versus $p=0.2$ for CD34⁺CD38⁺; for DNR, $p=0.05$ versus $p=0.5$).

To verify whether similar results can be obtained in a clonogenic assay, normal and AML CD34⁺ cells were exposed to lovastatin and/or cytostatic agents for 24 h and subsequently cultured in CFC medium. However, CD34⁺

Fig. 1 Cell survival of CD34⁺ and CD34⁻ normal (a; $n=7$) and AML cells (b; $n=17$) upon incubation with increasing lovastatin concentrations (25–100 μM). SEM are indicated

cells from six out of seven AML samples did not show any in vitro colony formation in the CFC assay. Normal CD34⁺ cells ($n=5$) showed an inhibition of 12% and 8% with 25 μM lovastatin or daunorubicin, respectively, which increased to 36% inhibition when both agents were used ($p=0.01$, data not shown). Comparable results were obtained cytarabin (28% versus 60%, $p=0.04$).

Given the observed variability in effects of lovastatin on the CD34⁺ AML cells, we questioned whether this might be explained by a difference in expression or modulation of downstream targets. Therefore, the ABCG1 and ABCA1 mRNA expression levels were studied both in the normal responder and abnormal responder AML group and compared to normal CD34⁺ cells. A nonsignificant higher ABCG1 mRNA expression was observed in the normal responder AML group compared to the abnormal responder AML group and normal CD34⁺ cells. In addition, the

lovastatin-induced ABCG1 mRNA expression was studied in the different groups. No statistically significant difference was found between normal CD34⁺ cells (0.4 fold down-regulation, range 0.29–0.36) versus the normal and abnormal AML responder group, 0.2 (range minus 0.16–0.53), and 0.5 fold (range 0.16–0.70) downregulation, respectively.

In vitro response pattern and clinical outcome

In view of the observed in vitro difference for statin sensitivity, we questioned whether these findings might correspond to patient characteristics or patient outcome. In the AML responder group, a normal or favorable cytogenetic pattern was found in 100% of the patients, while in the abnormal responder group, an unfavorable cytogenetic

Fig. 2 Effects of increasing concentrations of chemotherapeutic drugs on in vitro survival of subpopulations of AML cells. CD34⁺ (a) and CD34⁻ (b) AML cells of all patients ($n=17$) were incubated with lovastatin in combination with variable concentrations of daunorubicin (0.01–0.5 μM). SEM are indicated

Fig. 3 Effects of increasing concentrations of lovastatin in combination with chemotherapeutic drugs on in vitro survival of CD34⁺ (a–c) and CD34[−] (d–f) normal and AML cells. Three groups are demonstrated in a–f. Two AML patient groups (normal responders ($n=13$) and abnormal responders ($n=4$); in total, $n=17$) and a normal CD34^{+/−} group ($n=7$) are shown. SEM are indicated

pattern was present in 50% of the patients. In the responder group, the median peripheral blast cell count at presentation was $16.6 \times 10^9/L$ (range 2.2–128.3) and in the abnormal responder group was $81.4 \times 10^9/L$ (range 48.6–200, $p=0.02$). Based on these factors, three prognostic risk groups can be defined, i.e., favorable risk (favorable cytogenetics in combination with a peripheral blood blast cell count $<20 \times 10^9/L$ at diagnosis), poor risk (those with poor-risk cytogenetics), and intermediate risk group (all others) [18, 19]. In the responder group, 100% of the patients belonged to the good or intermediate risk group while in the abnormal responder group, this value was only 50% (Table 1). These findings correlated with treatment results. Complete remission on intensive chemotherapy was

attained in 91% of the patients in the AML normal responder group with an EFS of 12.5 months (range 1–37). In the abnormal responders, CR was attained in 17% of the patients with a EFS of 0 month (range 0–15).

Discussion

In the present study, we analyzed the in vitro effect of lovastatin on the survival of AML subpopulations in the absence or presence of conventional applied chemotherapeutics. So far, in vitro studies on statins in AML have been focused on the total AML cell population [6–9]. However, information of well-defined subpopulations

Fig. 4 Effects of increasing concentrations of lovastatin in combination with chemotherapeutic drugs on in vitro survival of sorted $CD34^+CD38^-$ (a, b) and $CD34^+CD38^+$ (c, d) AML cells. Two groups are shown: normal responders ($n=4$) and abnormal responders ($n=3$). SEM are indicated

might provide additional information especially regarding the primitive $CD34^+$ AML cells that are responsible for the ongoing in vivo propagation [19, 20]. By studying two different subpopulations of AML cells (primitive $CD34^+$ cells and more mature $CD34^-$ cells), we were able to demonstrate a significant difference in lovastatin susceptibility. The inhibitory effects of lovastatin were especially observed on the primitive $CD34^+$ subpopulation. The effects on the more mature $CD34^-$ subpopulation were less pronounced, suggesting that the primitive cell fraction is more dependent on cholesterol synthesis, which is in line with the high expression of ABC transporters in these cells. In addition, there were increased although variable inhibitory effects by the combined use of lovastatin and cytotoxic agents in both the AML and normal $CD34^+$ cells. Interestingly, we found that some AML $CD34^+$ cells followed a response pattern similar to normal $CD34^+$ cells while 35% of the AML $CD34^+$ cells showed no change. This could not be reversed in a number of cases by co-exposure the cells to chemotherapeutic agents. Interestingly, the distinction between normal and abnormal responders for the AML $CD34^+$ fraction was most pronounced in the $CD34^+CD38^-$ cell fraction, a population that is enriched for leukemic stem cells [21]. The difference in responsiveness between the

normal- and abnormal-responder AML groups could not be explained by a difference in the percentage of AML $CD34^+$ cells. In addition, no difference in susceptibility for lovastatin-mediated upregulation of ABCG1 mRNA was observed which is a major downstream target of lovastatin. It is more conceivable that the difference is linked to additional cellular characteristics of the AML cells belonging to both groups. Patient characteristics demonstrated that the abnormal responder group had a significant higher peripheral blast cell count at presentation. Moreover, the group was characterized by a high frequency of poor-risk cytogenetic abnormalities. The cause of the difference in susceptibility is not resolved but might be linked to differences in the farnesylation or geranylgeranylation pathways that are also affected by lovastatin [3, 6]. It has been demonstrated that statins disrupt the localization and function of isoprenylated molecules in the cell membrane [3, 6]. This change will result in an altered GTPase function of the Ras or Rho signaling that has an important impact on cellular processes such as proliferation and survival [22, 23]. Alternatively, the difference may be linked to an altered expression of anti-apoptotic proteins between both AML groups. It has been described that overexpression of Bcl-xL protects against statin-induced apoptosis in murine

tubular cells [24]. Both of these possible explanations are currently under evaluation.

In summary, these results demonstrate distinct differences in lovastatin susceptibility between CD34⁺ and CD34[−] in the normal and leukemic counterpart and between different AML subtypes. Additional studies will clarify whether the described in vitro assay can be used for in vivo identification of patients that might benefit from application of statins in combination with chemotherapeutic agents.

Acknowledgments We thank A.T.J. Wierenga for his help with the cell viability assay. This work was supported by a grant of the Dutch Cancer Society (RUG 2006-3580).

References

- Peeters SD, van der Kolk DM, de Haan G, Bystrykh L, Kuipers F, de Vries EG et al (2006) Selective expression of cholesterol metabolism genes in normal CD34⁺CD38[−] cells with a heterogeneous expression pattern in AML cells. *Exp Hematol* 34:622–630 doi:10.1016/j.exphem.2006.01.020
- de Grouw EP, Raaijmakers MH, Boezeman JB, van der Reijden BA, van de Locht LT, de Witte TJ et al (2006) Preferential expression of a high number of ATP binding cassette transporters in both normal and leukemic CD34⁺. *Leukemia* 20:750–754 doi:10.1038/sj.leu.2404131
- Wong J, Quinn CM, Brown AJ (2004) Statins inhibit synthesis of an oxysterol ligand for the liver x receptor in human macrophages with consequences for cholesterol flux. *Arterioscler Thromb Vasc Biol* 24:2365–2371 doi:10.1161/01.ATV.0000148707.93054.7d
- Li HY, Appelbaum FR, Willman CL, Zager RA, Banker DE (2003) Cholesterol-modulating agents kill acute myeloid leukemia cells and sensitize them to therapeutics by blocking adaptive cholesterol responses. *Blood* 101:3628–3634 doi:10.1182/blood-2002-07-2283
- Banker DE, Mayer SJ, Li HY, Willman CL, Appelbaum FR, Zager RA (2004) Cholesterol synthesis and import contribute to protective cholesterol increments in acute myeloid leukemia cells. *Blood* 104:1816–1824 doi:10.1182/blood-2004-01-0395
- Dimitroulakos J, Nohynek D, Backway KL, Hedley DW, Yeger H, Freedman MH et al (1999) Increased sensitivity of acute myeloid leukemias to lovastatin-induced apoptosis: a potential therapeutic approach. *Blood* 93:1308–1318
- Wu J, Wong WW, Khosravi F, Minden MD, Penn LZ (2004) Blocking the Raf/MEK/ERK pathway sensitizes acute myelogenous leukemia cells to lovastatin-induced apoptosis. *Cancer Res* 64:6461–6468 doi:10.1158/0008-5472.CAN-04-0866
- Minden MD, Dimitroulakos J, Nohynek D, Penn LZ (2001) Lovastatin induced control of blast cell growth in an elderly patient with acute myeloblastic leukemia. *Leuk Lymphoma* 40:659–662 doi:10.3109/10428190109097663
- Stirewalt DL, Appelbaum FR, Willman CL, Zager RA, Banker DE (2003) Mevastatin can increase toxicity in primary AMLs exposed to standard therapeutic agents, but statin efficacy is not simply associated with ras hotspot mutations or overexpression. *Leuk Res* 27:133–145 doi:10.1016/S0145-2126(02)00085-1
- Kornblau SM, Banker DE, Stirewalt D, Shen D, Lemker E, Verstovsek S et al (2007) Blockade of adaptive defensive changes in cholesterol uptake and synthesis in AML by the addition of pravastatin to idarubicin + high dose Ara-C: a phase 1 study. *Blood* 109:2999–3006
- van Gosliga D, Schepers H, Rizo A, van der Kolk D, Vellenga E, Schuringa JJ (2007) Establishing long-term cultures with self-renewing acute myeloid leukemia stem/progenitor cells. *Exp Hematol* 35:1538–1549 doi:10.1016/j.exphem.2007.07.001
- Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J et al (1999) World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting—Airlie House, Virginia, November 1997. *J Clin Oncol* 17:3835–3849
- Schepers H, van Gosliga D, Wierenga AT, Eggen BJ, Schuringa JJ, Vellenga E (2007) STAT5 is required for long-term maintenance of normal and leukemic human stem/progenitor cells. *Blood* 110:2880–2888 doi:10.1182/blood-2006-08-039073
- SPSS for Windows, Rel. 14.0.0. 2005. Chicago: SPSS Inc.
- Lowenberg B, Boogaerts MA, Daenen SM, Verhoef GE, Hagenbeek A, Vellenga E et al (1997) Value of different modalities of granulocyte-macrophage colony-stimulating factor applied during or after induction therapy of acute myeloid leukemia. *J Clin Oncol* 15:3496–3506
- Ossenkoppele GJ, Graveland WJ, Sonneveld P, Daenen SM, Biesma DH, Verdonck LF et al (2004) The value of fludarabine in addition to ARA-C and G-CSF in the treatment of patients with high-risk myelodysplastic syndromes and AML in elderly patients. *Blood* 103:2908–2913 doi:10.1182/blood-2003-07-2195
- Daenen SM, de Wolf JThM, Vellenga E, van Imhoff GW, van den Berg-de Ruitter E, Smit JW (2001) 6-Mercaptopurine, still valuable for the palliative treatment of acute myeloid leukemia. *Hematol* 6:231–40
- Breems DA, Boogaerts MA, Dekker AW, van Putten WL, Sonneveld P, Huijgens PC et al (2005) Autologous bone marrow transplantation as consolidation therapy in the treatment of adult patients under 60 years with acute myeloid leukaemia in first complete remission: a prospective randomized Dutch-Belgian Haemato-Oncology Co-operative Group (HOVON) and Swiss Group for Clinical Cancer Research (SAKK) trial. *Br J Haematol* 128:59–65 doi:10.1111/j.1365-2141.2004.05282.x
- Guan Y, Gerhard B, Hogge DE (2003) Detection, isolation, and stimulation of quiescent primitive leukemic progenitor cells from patients with acute myeloid leukemia (AML). *Blood* 101:3142–3149 doi:10.1182/blood-2002-10-3062
- de Jonge-Peters SD, Kuipers F, de Vries EG, Vellenga E (2007) ABC transporter expression in hematopoietic stem cells and the role in AML drug resistance. *Crit Rev Oncol Hematol* 62:214–226 doi:10.1016/j.critrevonc.2007.02.003
- Bonnet D, Dick JE (1997) Human acute myeloid leukaemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 3:730–737 doi:10.1038/nm0797-730
- Zhang FL, Casey PJ (1996) Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem* 65:241–269 doi:10.1146/annurev.bi.65.070196.001325
- Du W, Prendergast GC (1999) Geranylgeranylated RhoB mediates suppression of human tumor cell growth by farnesyltransferase inhibitors. *Cancer Res* 59:5492–5496
- Blanco-Colio LM, Justo P, Daehn I, Lorz C, Ortiz A, Egido J (2003) Bcl-xL overexpression protects from apoptosis induced by HMG-CoA reductase inhibitors in murine tubular cells. *Kidney Int* 64:181–191 doi:10.1046/j.1523-1755.2003.00080.x