Low-density lipoprotein receptor (LDLR) is an independent adverse prognostic factor in acute myeloid leukaemia

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Summary

The low-density lipoprotein receptor (LDLR) is a membrane receptor that mediates the endocytosis of low-density lipoprotein (LDL). Uptake of LDL has been proposed to contribute to chemotherapy resistance of acute myeloid leukaemia (AML) cell lines in vitro. In the present study, we analysed LDLR expression and survival using bone marrow biopsies from 187 intensively treated patients with AML. Here, increasing LDLR expression was associated with decreasing overall (58–4%, 44–2%, and 24–4%; \( P = 0.0018 \)), as well as event-free survival (41–7%, 18–1%, and 14–3%; \( P = 0.0077 \)), and an increasing cumulative incidence of relapse (33–9%, 55–1%, and 71–4%; \( P = 0.0011 \)). Associations of LDLR expression with survival were confirmed in 557 intensively treated patients from two international validation cohorts. In the analytic and validation cohorts, LDLR expression remained associated with outcome in multivariable regression analyses including the European LeukemiaNet genetic risk classification. Thus, LDLR predicts outcome of patients with AML beyond existing risk factors. Furthermore, we found low expression levels of LDLR in most healthy tissues, suggesting it as a promising target for antibody-based pharmacodelivery approaches in AML.

Keywords: acute myeloid leukaemia, LDLR, prognostic impact, novel targets, tissue microarray.

Introduction

The low-density lipoprotein receptor (LDLR) is a cell-surface glycoprotein that mediates the endocytosis of cholesterol-rich low-density lipoprotein (LDL). Elevated LDLR expression has been described for some solid tumours and uptake of LDL has been linked to tumour progression in vivo.2–4 Interestingly, AML cells have a higher LDL uptake than normal mononuclear blood cells and LDL is an important supplement for cultivating AML cells in vitro.5–9 Furthermore, uptake of LDL by AML cell lines has been proposed to contribute to chemotherapy resistance in vitro.10

Given these preclinical observations, we aimed to investigate the clinical role of LDLR expression in AML. We demonstrate that LDLR is expressed in primary samples from patients with AML and that its expression is inversely associated with patient outcome independent from established risk factors. Protein levels of LDLR are higher in patients with secondary AML (sAML) compared to de novo AML. The low protein expression of LDLR in healthy human organs makes it a promising target for novel pharmacodelivery approaches in AML.

Patients and methods

Patients, samples and treatment

Protein expression of LDLR was analysed in pre-therapeutic bone marrow (BM) trephines from 187 intensively treated patients with AML receiving intensive chemotherapy with cytarabine and daunorubicin at the University Hospital Münster (UKM) between 2006 and 2016 (Table I).11 The study was approved by the regional Institutional Review Board (2016-382-f-S and 2016-654-f-S). A cohort of intensively treated patients with de novo AML (\( n = 400 \)) from the Dutch-Belgian Hematology Oncology Cooperative Group (HOVON)12,13 and The Cancer Genome Atlas (TCGA) AML cohort (\( n = 157 \))14 served as validation sets. Patients with acute promyelocytic leukaemia or myelodysplastic syndromes were excluded from all cohorts. A study profile is shown in...
Table 1. Pre-treatment characteristics of 187 patients with AML in the UKM analytic cohort, categorised as LDLR<sup>low</sup>, LDLR<sup>int</sup> and LDLR<sup>high</sup>.

| Variable | LDLR<sup>low</sup> | LDLR<sup>int</sup> | LDLR<sup>high</sup> | P |
|----------|------------------|------------------|------------------|---|
| N        | 42               | 96               | 49               |   |
| Age, years, median (range) | 57-1 (26-5–74-9) | 54-6 (20-4–79-7) | 57-6 (36-4–84-4) | 0.64§ |
| Sex, n (%) |                   |                  |                  | 0.84§ |
| Male     | 26 (61-9)        | 52 (54-2)        | 31 (63-3)        |   |
| Female   | 16 (38-1)        | 44 (45-8)        | 18 (36-7)        |   |
| AML type, n (%) |               |                  |                  | 0.0086‡ |
| De novo  | 28 (66-7)        | 58 (60-4)        | 19 (38-8)        |   |
| sAML     | 8 (19-0)         | 31 (32-3)        | 26 (53-1)        |   |
| tAML     | 6 (14-3)         | 7 (7-3)          | 4 (8-2)          |   |
| FAB, n (%) |                   |                  |                  | 0.83‡ |
| M0       | 4 (10-0)         | 5 (5-4)          | 1 (2-7)          |   |
| M1       | 3 (7-5)          | 13 (14-1)        | 3 (8-1)          |   |
| M2       | 4 (10-0)         | 20 (21-7)        | 9 (24-3)         |   |
| M4       | 12 (30-0)        | 27 (29-3)        | 11 (29-7)        |   |
| M5       | 13 (32-5)        | 20 (21-7)        | 10 (27-0)        |   |
| M6       | 3 (7-5)          | 5 (5-4)          | 2 (5-4)          |   |
| M7       | 1 (2-5)          | 2 (2-2)          | 1 (2-7)          |   |
| WBC, ×10<sup>9</sup>/l, median (range) | 13-2 (0-4–242-6) | 9.4 (0-8–289-5) | 15-9 (0-7–420-2) | 0.28§ |
| LDH level, u/l, median (range) | 391-5 (155-0–2451-0) | 445-5 (138-0–3354-0) | 473-0 (156-0–4463-0) | 0.46§ |
| Hb, g/l, median (range) | 86 (46-119) | 89 (38-157) | 89 (36-140) | 0.36§ |
| Platelets, ×10<sup>9</sup>/l, median (range) | 41-5 (7-0–320-0) | 61-5 (6-0–475-0) | 62-0 (6-0–243-0) | 0.19§ |
| BM blasts, %, median (range) | 70-0 (23-0–95-0) | 70-0 (10-0–90-0) | 60-0 (15-0–90-0) | 0.11§ |
| Cytogenetics*, n (%) |                   |                  |                  |   |
| t(8;21)  | 0 (0-0)          | 4 (4-2)          | 1 (2-0)          | 0.60§ |
| inv(16)/t(16;16) | 1 (2-4) | 5 (5-2) | 0 (0-0) | 0.47§ |
| normal   | 23 (54-8)        | 47 (50-0)        | 23 (47-9)        | 0.52§ |
| t(9;11)  | 1 (2-4)          | 1 (1-1)          | 0 (0-0)          | 0.28§ |
| t(6;9)   | –                | –                | –                |   |
| t(9;22)  | 0 (0-0)          | 0 (0-0)          | 1 (2-0)          | 0.17§ |
| t(8;11q23) | 0 (0-0) | 4 (4-3) | 1 (2-1) | 0.59§ |
| inv(3)/t(3;3) | 2 (4-8) | 2 (2-1) | 0 (0-0) | 0.12§ |
| -5/del(5q) | 2 (4-8) | 6 (6-4) | 2 (4-2) | 0.88§ |
| -7       | 2 (4-8)          | 5 (5-3)          | 3 (6-2)          | 0.75§ |
| -17/abn(17p) | 2 (4-8) | 2 (2-1) | 2 (4-2) | 0.91§ |
| Complex  | 5 (11-9)         | 12 (12-8)        | 8 (16-7)         | 0.50§ |
| Monosomal| 3 (7-1)          | 3 (3-2)          | 5 (10-4)         | 0.47§ |
| Other    | 8 (19-0)         | 13 (13-8)        | 12 (25-0)        | 0.42§ |
| FLT3-ITD, n (%) |               |                  |                  |   |
| Present  | 5 (12-2)         | 14 (14-6)        | 7 (14-3)         |   |
| Absent   | 36 (87-8)        | 82 (85-4)        | 42 (85-7)        |   |
| NPM1, n (%) |                   |                  |                  | 0.22§ |
| Mutated  | 11 (26-8)        | 29 (30-5)        | 8 (16-3)         |   |
| Wild type| 30 (73-2)        | 66 (69-5)        | 41 (83-7)        |   |
| NPM1<sup>mut</sup>/FLT3-ITD<sup>mut</sup>, n (%) |               |                  |                  | 0.70‡ |
| NPM1<sup>mut</sup>/FLT3-ITD<sup>del</sup> | 8 (19-5) | 20 (21-1) | 5 (10-2) |   |
| NPM1<sup>mut</sup>/FLT3-ITD<sup>del</sup> | 3 (7-3) | 9 (9-5) | 3 (6-1) |   |
| NPM1<sup>mut</sup>/FLT3-ITD<sup>del</sup> | 28 (68-3) | 61 (64-2) | 37 (75-5) |   |
| NPM1<sup>mut</sup>/FLT3-ITD<sup>del</sup> | 2 (4-9) | 5 (5-3) | 4 (8-2) |   |
| Cyto genetic and molecular risk†, n (%) |               |                  |                  | 0.36§ |
| Favourable| 7 (16-7)         | 24 (25-5)        | 4 (8-3)          |   |
| Intermediate| 26 (61-9)       | 46 (48-9)        | 32 (66-7)        |   |
| Adverse  | 9 (21-4)         | 24 (25-5)        | 12 (25-0)        |   |
Table 1. (Continued)

| Variable         | LDLR    |          |          |          | P       |
|------------------|---------|----------|----------|----------|---------|
|                  | Low     | Intermediate | High    |          |         |
| Allogeneic SCT   |         |           |          |          | 0·0055  |
| None             | 26 (61·9) | 43 (45·3) | 16 (34·0) |          |         |
| CR1              | 9 (21·4)  | 14 (14·7) | 11 (23·4) |          |         |
| >CR1             | 7 (16·7)  | 38 (40·0) | 20 (42·6) |          |         |

AML, acute myeloid leukaemia; BM, bone marrow; FAB, French-American-British classification; FLT3-ITD, internal tandem duplication of the FLT3 gene; Hb, haemoglobin; LDH, lactate dehydrogenase; NPM1, nucleophosmin-1; sAML, secondary AML; tAML, therapy-related AML; WBC, white blood cell count.

Significant P value marked in bold.

* According to European LeukemiaNet 2010 guidelines (information on CEBPA mutational status not available).
† Fisher’s exact test.
‡ Jonckheere–Terpstra test.
¶ Mantel-Haenszel test.

Figure S1. Cytogenetic and molecular risk was classified according to the European LeukemiaNet (ELN) genetic risk classification.15,16

Procedures

Tissue microarrays (TMAs) were generated from formalin-fixed paraffin-embedded BM trephines as described.11,17–20 Two representative 1-mm cores from areas of leukaemic infiltration were arrayed per sample. Immunohistochemical staining was performed as described.11,21,22 Briefly, following de-paraffinisation and heat-induced epitope unmasking, 4-μm tissue sections were incubated with a primary anti-LDLR antibody (ab30532; Abcam, Cambridge, UK), followed by suitable secondary and tertiary antibodies (Dako, Carpinteria, CA, USA). Immunoreactions were visualised with a monoclonal alkaline phosphatase-anti-alkaline phosphatase (APAAP) complex and a fuchsin-based substrate-chromogen system (Dako). Counterstaining was performed with Mayer’s haemalum (Merck). All slides were examined using a Nikon Eclipse 50i microscope equipped with a Nikon DS-2Mv. Immunoreactivity (1 = no/weak, 2 = moderate, 3 = strong staining intensity) and percentage of stained blasts at each immunoreactivity level were assessed by two investigators (M.F. and S.E.), who were blinded towards patient characteristics and outcome. H-scores combining intensity of staining and percentage of stained blasts were calculated using the following formula: H-score = 1 × (percentage of blasts positive at immunoreactivity level 1) + 2 × (percentage of blasts positive at immunoreactivity level 2) + 3 × (percentage of blasts positive at immunoreactivity level 3).11,23,24

Gene expression from the HOVON cohort has previously been generated with Affymetrix HG-U133 Plus 2.0 chips and has been published previously.12,13 Gene expression data and clinical information are publicly available (GSE6891). Expression data were pre-processed as described.11,13 TCGA RNAsseq and clinical data have been published previously and were downloaded from the cBioPortal.14

The LDLR protein expression data were downloaded from the publicly available data repository Human Proteome Map.25 Expression values were obtained from histologically normal samples on mass spectrometers and are given as averaged normalised spectral counts.

Statistical analyses

To define LDLR expression status, the analytic cohort was divided into quartiles according to LDLR expression values (H-scores) and subsequently trichotomised into three groups with low (quartile 1 [Q1]), intermediate (Q2/Q3) and high (Q4) expression. Q2 and Q3 were combined into an intermediate expression group as they displayed identical outcome. Identical quartile-based stratification was subsequently used for each gene expression validation set.

Time-to-event variables and complete remission (CR) were defined as described.11–14 Follow-up time was calculated by the reverse Kaplan–Meier method. Survival probabilities and incidences were determined using the Kaplan–Meier and the Aalen–Johansen estimator, compared using log-rank and Gray’s test, respectively, and are given at 5 years. Clinical and molecular baseline variables were compared between LDLR expression groups using Mantel–Haenszel, Fisher’s exact or Jonckheere–Terpstra test.

Multivariable Cox proportional hazards models were generated to assess statistical significance of prognostic factors with respect to overall (OS) and event-free survival (EFS). Likewise, multivariable Fine–Gray models were computed to evaluate cumulative incidence of relapse (CIR) and multivariable logistic regression models to assess achievement of CR. Besides LDLR expression, age, AML type and cytogenetic and molecular risk factors were entered in the multivariable models. The additional value of LDLR expression to the
**Results**

The median (interquartile range [IQR]) follow-up time for the UKM patients was 5.21 (3.90–7.27) years. Patients in the highest quartile of LDLR expression (LDLR<sup>high</sup>; H-score ≥200) had a significantly inferior OS (24.4%) than patients in the intermediate quartiles (LDLR<sup>int</sup>; H-score 125–199; 44.2%) or lowest quartile (LDLR<sup>low</sup>; H-score <125; 58.4%; \( P = 0.0018 \); Fig 1A). Likewise, EFS was inferior in patients who were LDLR<sup>high</sup> (14.3%) compared to LDLR<sup>int</sup> (18.1%) or LDLR<sup>low</sup> (41.79%; \( P = 0.0077 \); Fig 1B). We found no significant differences between CR rates when comparing LDLR<sup>low</sup> (64.3%), LDLR<sup>int</sup> (57.3%) and LDLR<sup>high</sup> groups (57.1%, \( P = 0.72 \)). However, there was a significantly higher CIR in patients in first CR (CR1) who were LDLR<sup>high</sup> (71.4%) compared to LDLR<sup>int</sup> (55.1%) or LDLR<sup>low</sup> (33.99%; \( P = 0.0011 \); Fig 1C). The median OS was 0.93, 2.31 and 7.84 years, median EFS was 0.51, 0.85 and 1.03 years, and median CIR was 0.79, 1.69 years and not reached for high-, intermediate- and low-LDLR expression, respectively. As a continuous variable, an increase of LDLR expression by 50 H-score units was associated with an increased risk of death (hazard ratio [HR] 1.28, 95% confidence interval [CI] 1.07–1.54; \( P = 0.0078 \)), experiencing an EFS event (HR 1.25, 95% CI 1.07–1.46; \( P = 0.0055 \)) and relapse (HR 1.45, 95% CI 1.15–1.81; \( P = 0.0014 \)), but not with the likelihood of achieving a CR (odds ratio [OR] 0.94, 95% CI 0.70–1.27; \( P = 0.69 \)).

Baseline characteristics of the 187 patients from the UKM analytic cohort by trichotomised LDLR expression are listed in Table I. Patients with high expression of LDLR more frequently had sAML than de novo or therapy-related AML (tAML) (\( P = 0.0086 \)). Rates of allogeneic haematopoietic stem-cell transplantation (alloHSCT) were higher in patients who were LDLR<sup>high</sup> than LDLR<sup>low</sup> (66.0% vs. 38.1%, \( P = 0.0055 \)) due to more frequent transplantations beyond CR1. We found no associations with age, sex, French-American-British classification (FAB) type, white blood cell count, lactate dehydrogenase, and molecular or cytogenetic risk. In addition, we found no significant interaction of any of the baseline variables with OS, EFS or CIR according to LDLR expression in exploratory subgroup analyses Fig 2.

When adjusting for age, AML type and cytogenetic and molecular risk (Table II), a per 50-unit increase in LDLR expression was associated with an increasing risk of death (HR 1.26, 95% CI 1.04–1.54; \( P = 0.021 \)) and experiencing an event (HR 1.21, 95% CI 1.02–1.44; \( P = 0.026 \)). While there was no association of LDLR expression and the likelihood to achieve a CR (HR 1.01, 95% CI 0.72–1.42; \( P = 0.94 \)), there was a significantly increased risk of relapse after CR1 with increasing LDLR expression (HR 1.37, 95% CI 1.05–1.79; \( P = 0.020 \)). LDLR expression significantly improved the multivariable models beyond the standard clinical risk factors for OS (\( P = 0.024 \)), EFS (\( P = 0.029 \)) and CIR (\( P = 0.023 \)). We found no relevant collinearity among predictors with a median (range) variance inflation factor of 1.43 (1.11–3.23).
LDLR expression also remained significantly associated with OS, EFS and CIR when included as a categorical variable Table SI.

To validate the associations of LDLR expression with outcome, we interrogated LDLR gene expression in two independent cohorts. In the HOVON cohort, increasing levels of

Fig 2. LDLR protein expression and overall survival (A), event-free survival (B) and incidence of relapse (C) by subgroups. Hazard ratios (HRs) and confidence intervals for continuous LDLR expression are shown. The \( P \) values are for interaction of HRs by subgroups. \( ^{\text{§}}P \) heterogeneity; \( ^{\text{¶}}P \) trend.
Table 2. Multivariable regression analyses in the UKM analytic cohort.

| Variables in final models | OR/HR  | 95% CI | P       |
|---------------------------|--------|--------|---------|
| **Complete remission**    |        |        |         |
| Age                       |        |        |         |
| Per 10-year increase      | 0.91   | 0.72–1.17 | 0.47    |
| ELN 2010†                 |        |        |         |
| Intermediate vs. favourable risk | 0.46 | 0.18–1.19 | 0.11    |
| Adverse vs. favourable risk | 2.0   | 0.06–6.65 | 0.0071  |
| Type of AML               |        |        |         |
| sAML vs. de novo          | 0.66   | 0.29–1.53 | 0.33    |
| tAML vs. de novo          | 1.09   | 0.34–3.49 | 0.49    |
| LDLR expression           |        |        |         |
| Per 50-H-score increase   | 1.01   | 0.72–1.42 | 0.93    |
| **Overall survival**      |        |        |         |
| Age                       |        |        |         |
| Per 10-year increase      | 1.35   | 1.14–1.59 | 0.0006  |
| ELN 2010†                 |        |        |         |
| Intermediate vs. favourable risk | 1.37 | 0.73–2.57 | 0.33    |
| Adverse vs. favourable risk | 2.88  | 1.38–6.01 | 0.0049  |
| Type of AML               |        |        |         |
| sAML vs. de novo          | 1.27   | 0.76–2.10 | 0.36    |
| tAML vs. de novo          | 1.27   | 0.63–2.56 | 0.51    |
| LDLR expression           |        |        |         |
| Per 50-H-score increase   | 1.26   | 1.04–1.54 | 0.021   |
| **Event-free survival**   |        |        |         |
| Age                       |        |        |         |
| Per 10-year increase      | 1.37   | 1.20–1.58 | 0.0001  |
| ELN 2010†                 |        |        |         |
| Intermediate vs. favourable risk | 1.24 | 0.77–2.01 | 0.38    |
| Adverse vs. favourable risk | 1.68  | 0.92–3.06 | 0.093   |
| Type of AML               |        |        |         |
| sAML vs. de novo          | 1.14   | 0.74–1.77 | 0.55    |
| tAML vs. de novo          | 1.25   | 0.70–2.24 | 0.45    |
| LDLR expression           |        |        |         |
| Per 50-H-score increase   | 1.21   | 1.02–1.44 | 0.026   |
| **Cumulative incidence of relapse** | |        |         |
| Age                       |        |        |         |
| Per 10-year increase      | 1.58   | 1.26–1.99 | 0.0001  |
| ELN 2010†                 |        |        |         |
| Intermediate vs. favourable risk | 1.40 | 0.70–2.80 | 0.34    |
| Adverse vs. favourable risk | 2.42  | 0.92–6.35 | 0.072   |
| Type of AML               |        |        |         |
| sAML vs. de novo          | 0.80   | 0.39–1.64 | 0.35    |
| tAML vs. de novo          | 0.71   | 0.27–1.85 | 0.48    |
| LDLR expression           |        |        |         |
| Per 50-H-score increase   | 1.37   | 1.05–1.79 | 0.020   |

Odds ratios (OR) greater or less than 1.0 indicate higher or lower CR rates, respectively, for the first category listed. Hazard ratios (HR) greater or less than 1.0 indicate an increased or decreased risk, respectively, of an event for the higher values of the continuous variables and the first category listed of the categorical variables. Significant P values are marked in bold.

AML, acute myeloid leukaemia; ELN, European LeukemiaNet; LDLR, low-density lipoprotein receptor; sAML, secondary AML; tAML, therapy-related AML.

*P value from the nested likelihood-ratio test.

†Genetic risk groups according to ELN 2010 definitions (information on CEBPA mutational status not available).

LDLR mRNA expression were associated with decreasing OS (47.8%, 38.2% and 31.0%; P = 0.014; Fig 3A) and EFS (35.9%, 30.6% and 24.0%; P = 0.032; Fig 3C). A per-unit increase in LDLR expression remained associated with an increased risk of death (HR 1.55, 95% CI 1.17–2.07; P = 0.0027) or experiencing an EFS event (HR 1.46, 95% CI 1.11–1.92; P = 0.0067) after adjustment for age, ELN 2010 genetic risk and additional sex combs like-1 (ASXL1)
mutational status (Table SII). Expression of LDLR significantly improved the multivariable models beyond the standard clinical risk factors for OS ($P = 0.0036$) and EFS ($P = 0.0082$). In the TCGA cohort, survival decreased with increasing LDLR expression levels: OS (38\% vs. 23\% vs. 11\%; $P = 0.0016$; Fig 3B) and EFS (27\% vs. 17\% vs. 10\%; $P = 0.0018$; Fig 3D). A per 500-unit increase in LDLR expression remained associated with an increased risk of death (HR 1.16, 95\% CI 1.04–1.30; $P = 0.0079$) or experiencing an EFS event (HR 1.13; 95\% CI 1.02–1.26; $P = 0.020$) after adjustment for age and ELN 2017 genetic risk (Table SIII). Expression of LDLR significantly improved the multivariable models beyond the standard clinical risk factors for OS ($P = 0.013$) and EFS ($P = 0.027$). Multivariable regression analyses for OS and EFS with LDLR as a categorical variable can be found in Tables SIV and S5. Data on CR and CIR were not available for both cohorts.

LDLR was expressed across all morphological subgroups and cytogenetic risk groups of AML (Fig 4B). We found an elevated expression in samples from patients with sAML compared to de novo AML (median H-score 145 vs. 190, $P = 0.0003$; Fig 4B). Expression of LDLR was lower in normal than in leukaemic BM (Fig 4A). Furthermore, LDLR expression was analysed in normal tissues in silico using proteomic data from the Human Proteome Database (Figure 4C). In agreement with our histological data, there was no expression of LDLR in haematopoietic cells, as measured by mass spectrometry, and high expression values of LDLR were only found in fetal tissues and the adrenal glands.

**Discussion**

We report an inverse association of increasing LDLR expression levels with outcome in several cohorts of intensively treated patients with AML. LDLR expression levels were higher in patients with sAML compared to de novo AML. The prognostic effect was observed across different genetic risk groups and LDLR remained significantly associated with prognosis when genetic risk classification was included in the multivariable analyses. In any case, standardisation of the methods used to determine the LDLR expression levels is necessary, before quantitative measurement of LDLR expression can be used in the clinical setting. Immunohistochemical staining of LDLR on BM sections to identify patients with high LDLR expression by H-scoring, as performed in the present study, may be an option for clinical translation. However, this should be validated within the framework of a prospective clinical trial.
Interfering with cholesterol synthesis, import and metabolism has been described as a treatment strategy across various cancer entities. In fact, elevated expression of LDLR has been involved in the progression of breast cancer in vivo and its silencing results in decreased tumour growth. Furthermore, downregulation of LDLR has been shown to increase sensitivity of solid tumours towards chemotherapy and pharmaceutical targeting of LDLR through liver X receptor (LXR) agonists induces tumour cell death in vivo. Temporary functional disruption of LDLR is unlikely to cause severe harm in humans considering the relatively late consequences of the congenital loss of functional LDLR in patients with familial hypercholesterinaemia. However, vulnerabilities of healthy organs to a combination of LDLR suppression with chemotherapy will have to be determined. A combination of agents that target different aspects of the cholesterol homeostasis pathways might be necessary to avoid escape mechanisms.

Another way of exploiting the upregulation of LDLR in malignant diseases for therapeutic targeting has been proposed through formulation of cytotoxic drugs into apolipoprotein E-expressing liposomes. Furthermore, protein-free microemulsions with a lipid structure resembling that of LDL have been shown to acquire endogenous apolipoprotein E after intravenous injection and target LDLR expressing malignant cells. Thus, such lipid emulsions might be used as vehicles that carry cytotoxic molecules to LDLR-expressing AML cells. In fact, a liposomal formulation of daunorubicin and cytarabine (CPX-351) has recently been approved for the treatment of sAML. Scavenger receptors and LDLR have been proposed as potential receptors for the uptake of CPX-351 into AML cells. The higher expression levels of LDLR that we observed in sAML compared to de novo AML might contribute to the treatment benefit of CPX-351, which was preferentially seen amongst patients with sAML. Targeting LDLR with antibodies that are coupled to a potent cytotoxic drug might represent a more specific concept for the targeting of sAML.

Antibody–drug conjugates are increasingly being used for the treatment of malignancies in the clinic and the first antibody–drug conjugate has recently been approved for the treatment of CD33+ de novo AML. However, there is no antibody available for the treatment of sAML to date and patients with sAML have a particularly poor prognosis. We found an upregulation of LDLR protein expression in AML compared to normal BM, with particularly high expression levels in patients with sAML. In addition, there were low protein expression values in healthy organs except for the brain, kidney and placenta.

![Image of LDLR expression in AML subgroups and normal human tissues](image)

Fig 4. LDLR protein expression in AML subgroups and normal human tissues. (A) Representative immunohistochemistry micrographs of LDLR expression in pre-therapeutic BM from an patient with AML with secondary AML (left), a patient with de novo AML (middle) and a healthy donor (right). Scale bars are 25 μm. (B) Distribution of LDLR H-scores in the complete cohort, by French-American-British classification (FAB) type, AML type and genetic risk group. Violin plots of H-scores including a boxplot with Tukey whiskers are shown. (C) Expression of LDLR by mass spectrometry in various healthy tissues according to the Human Proteome Map.
adrenal glands and some fetal tissues. Thus, LDLR might be an attractive target for the specific delivery of bioactive payloads, such as cytotoxic drugs, to the leukaemic blasts using antibody–drug conjugates, especially in the context of sAML. Bispecific T-cell engagers and chimeric antigen receptor T cells targeting LDLR on AML blasts represent further potential therapeutic applications. Interestingly, exposure of AML blasts to chemotherapy or 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors has been shown to increase the LDLR expression on leukaemic blasts. However, further analyses including biodistribution and in vivo targeting are necessary to determine the real effectiveness of these approaches and potential vulnerabilities of healthy tissues to anti-LDLR treatment in the absence and presence of these agents.

In conclusion, we identified LDLR expression as a novel prognostic factor in AML that is independent from established risk factors. The limited expression of LDLR in healthy organs renders it an attractive target for antibody-based therapies in AML. Further studies are necessary to determine the functional role of LDLR in AML and to confirm its potential as a therapeutic target.

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Conflict of interests

The authors declare no competing interests.

Author contributions

Linus Angenendt and Christoph Schliemann designed the study. Matthias Floeth, Sandra Elges, Wolfgang Hartmann, Christoph Schliemann and Linus Angenendt characterised the UKM cohort, performed and evaluated the TMA staining. Linus Angenendt performed statistical studies and analysed the data. Joachim Gerss and Christoph Schliemann contributed to data analysis. Matthias Floeth, Sandra Elges, Christoph Schliemann and Linus Angenendt wrote the manuscript. All authors interpreted the data and made the decision to submit the manuscript for publication.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Multivariable regression analyses in the UKM analytic cohort including LDLR expression as a categorical variable.

Table SII. Multivariable regression analyses in the HOVON cohort including LDLR expression as a continuous variable.

Table SIII. Multivariable regression analyses in the TCGA cohort including LDLR expression as a continuous variable.

Table SIV. Multivariable regression analyses in the HOVON cohort including LDLR expression as a categorical variable.

Table SV. Multivariable regression analyses in the TCGA cohort including LDLR expression as a categorical variable.

Figure S1. Flowchart of LDLR expression analysis in the analytic and validation cohorts. Patients with acute promyelocytic leukaemia (APL) or myelodysplastic syndrome (MDS) and patients with missing survival or LDLR expression data were excluded.

References

1. Joos H, Blacklow SC. Structure and physiologic function of the low-density lipoprotein receptor. Annu Rev Biochem. 2005;74:535–62.
2. Furuya Y, Sekine Y, Kato H, Miyazawa Y, Koike H, Suzuki K. Low-density lipoprotein receptors play an important role in the inhibition of prostate cancer cell proliferation by statins. Prostate Int. 2016;4:56–60.
3. Gallagher EJ, Zelenko Z, Neel RA, Antoniou IM, Rajan L, Kase N, et al. Elevated tumor LDLR expression accelerates LDL cholesterol-mediated breast cancer growth in mouse models of hyperlipidemia. Oncogene. 2017;36:6462–71.
4. Guillaumeond F, Bidaut G, Ouassim M, Servais S, Gourraud V, Olivares O, et al. Cholesterol uptake disruption, in association with chemotherapy, is a promising combined metabolic therapy for pancreatic adenocarcinoma. Proc Natl Acad Sci USA. 2015;112:2473–8.
5. Bhuiyan H, Masquelier M, Tatidis L, Gruber A, Paul C, Vitols S. Acute Myelogenous leukemia cells secrete factors that stimulate cellular LDL uptake via autocrine and paracrine mechanisms. Lipids. 2017;52:523–34.
6. Brabetz O, Alla V, Angenendt L, Schliemann C, Berdel WE, Arteaga MF, et al. RNA-guided CRISPR-Cas9 system-mediated engineering of acute myeloid leukemia mutations. Mol Ther Nucleic Acids. 2017;6:243–8.
7. Vitols S, Angelin B, Ericsson S, Gahtron G, Juliusson G, Masquelier M, et al. Uptake of low density lipoproteins by human leukemic cells in vivo: relation to plasma lipoprotein levels and possible relevance for selective chemotherapy. Proc Natl Acad Sci USA. 1990;87:2598–602.
8. Vitols S, Gahtron G, Ost A, Peterson C. Elevated low density lipoprotein receptor activity in leukemic cells with monocytic differentiation. Blood. 1984;63:1186–93.
9. Wenge DV, Felipe-Fumero E, Angenendt L, Schliemann C, Schmidt E, Schmidt LH, et al. MN1-Fli1 oncogenesis transforms murine hematopoietic progenitor cells into acute megakaryoblastic leukemia cells. Oncogenesis. 2015;4:e179.
10. Banker DE, Mayer SJ, Li HY, Willman CL, Appelbaum FR, Zager RA. Cholesterol synthesis and import contribute to protective cholesterol increments in acute myeloid leukemia cells. Blood. 2004;104:1816–24.
11. Angenendt L, Bormann E, Pahit C, Alla V, Görlich D, Braun L, et al. The neuropeptide receptor calcitonin receptor-like (CALCRL) is a potential therapeutic target in acute myeloid leukemia. Leukemia. 2019;33:2830–41.
12. Valk PJ, Verhaak RG, Beijen MA, Erpelink CA, van Waalwijk B, van Doorn-Khosrovani S, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med. 2004;350:1617–28.
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13. Verhaak RG, Wouters BJ, Erpelink CA, Abbas S, Verloove HB, Lughart S, et al. Prediction of molecular subtypes in acute myeloid leukemia based on gene expression profiling. *Haematologica.* 2009;94:131–4.

14. The Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med.* 2013;368:2059–74.

15. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood.* 2017;129:424–47.

16. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood.* 2010;115:453–74.

17. Angenendt L, Mikesch JH, Górlisch D, Busch A, Arnhold I, Rudack C, et al. Stromal collagen type VI associates with features of malignancy and predicts poor prognosis in salivary gland cancer. *Cell Oncol (Dordr).* 2018;41:517–25.

18. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton J, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med.* 1998;4:844–7.

19. Mikesch JH, Hartmann W, Angenendt L, Huber O, Schliemann C, Arteaga MF, et al. AAA+ ATPases Reptin and Pontin as potential diagnostic and prognostic biomarkers in salivary gland cancer - a short report. *Cell Oncol (Dordr).* 2018;41:455–62.

20. Mikesch JH, Schwammbach D, Hartmann W, Schmidt LH, Schliemann C, Angenendt L, et al. Reptin drives tumour progression and resistance to chemotherapy in nonsmall cell lung cancer. *Eur Respir J.* 2018;52:1701637.

21. Angenendt L, Reuter S, Kentrup D, Benk AS, Neumann F, Huve I, et al. An atlas of bloodstream-accessible bone marrow probes for site-directed therapy of acute myeloid leukemia. *Leukemia.* 2018;32:510–9.

22. Schliemann C, Guthbrodt KL, Kerkhoff A, Pohlen M, Wiebe S, Silling G, et al. Targeting interleukin-2 to the bone marrow stroma for therapy of acute myeloid leukemia relapsing after allogeneic hematopoietic stem cell transplantation. *Cancer Immunol Res.* 2015;3:547–56.

23. Rastgoo N, Pourabdollah M, Abdi J, Reece D, Chang H. Dysregulation of EZH2/miR-138 axis contributes to drug resistance in multiple myeloma by downregulating RBPMS. *Leukemia.* 2018;32:471–82.

24. Roemer MGM, Redd RA, Cader FZ, Pak CJ, Abdelrahman S, Ouyang J, et al. Major histocompatibility complex Class II and programmed Death Ligand 1 expression predict outcome after programmed Death 1 blockade in classic Hodgkin lymphoma. *J Clin Oncol.* 2018;36:942–50.

25. Kim MS, Pinto SM, Getnet D, Nirujiogi RS, Manda SS, Chaerkady R, et al. A draft map of the human proteome. *Nature.* 2014;509:575–81.

26. Guo D, Reinitz F, Yousef M, Hong C, Nathanson D, Akhavan D, et al. An LXR agonist promotes glioblastoma cell death through inhibition of an EGFRAKT/SREBP-1/LDLR-dependent pathway. *Cancer Discov.* 2011;1:442–56.

27. Sukhanova A, Gorin A, Serebriiskii IG, Gaitova L, Zheng H, Restifo N, et al. Targeting C4-demethylating genes in the cholesterol pathway sensitizes cancer cells to EGF receptor inhibitors via increased EGF receptor degradation. *Cancer Discov.* 2013;3:96–111.

28. Yamaguchi R, Perkins G, Hirota K. Targeting cholesterol with beta-cyclodextrin sensitizes cancer cells for apoptosis. *FEBS Lett.* 2015;589:4097–105.

29. Versluis AJ, Rump ET, Rensen PCN, van Berkel TJG, Bijsterbosch MK. Stable incorporation of a lipophilic daunorubicin prodrug into apolipoprotein E-exposing liposomes induces uptake of produg via low-density lipoprotein receptor in vivo. *J Pharmacol Exp Ther.* 1999;289:1–7.

30. Maranaho RC, Roland IA, Tofoloetto O, Ramirez JA, Goncalves RP, Mesquita CH, et al. Plasma kinetic behavior in hyperlipidemic subjects of a lipidic microemulsion that binds to low density lipoprotein receptors. *Lipids.* 1997;32:627–33.

31. Lancet JE, Uy GL, Cortes JE, Newell LF, Lin TL, Ritchie EK, et al. CPX-351 (cytarabine and daunorubicin) liposome for injection versus conventional cytarabine plus daunorubicin in older patients with newly diagnosed secondary acute myloid leukemia. *J Clin Oncol.* 2018;36:2684–92.

32. Lim WS, Tardi PG, Dos Santos N, Xie X, Fan M, Liboire BD, et al. Leukemia-selective uptake and cytotoxicity of CPX-351, a synergistic fixed-ratio cytarabine/daunorubicin formulation, in bone marrow xenografts. *Leuk Res.* 2010;34:1214–23.

33. Lancet JE, Cortes JE, Hogge DE, Tallman MS, Kvascivovsky T, Damon LE, et al. Phase 2 trial of CPX-351, a fixed 5:1 molar ratio of cytarabine/daunorubicin, vs. cytarabine/daunorubicin in older adults with untreated AML. *Blood.* 2014;123:3239–46.

34. Lambert J, Pautas C, Terre C, Raffoux E, Turlure P, Gaillot D, et al. Gemtuzumab ozogamicin for de novo acute myeloid leukemia: final efficacy and safety updates from the open-label, phase III ALFA-0701 trial. *Haematologica.* 2019;104:113–9.

35. Bäumer N, Appel N, Terheyden L, Buchholz F, Rössig C, Müller-Tidow C, et al. Antibody-coupled siRNA as an efficient method for in vivo miRNA knockdown. *Nat Protoc.* 2016;11:22–36.

36. Vitols S, Norgren S, Balussius G, Tatidis L, Luthman H. Multilevel regulation of low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression in normal and leukemic cells. *Blood.* 1994;84:2689–98.