Cancer Research

CD38 in Hairy Cell Leukemia Is a Marker of Poor Prognosis and a New Target for Therapy

Nicolas Poret[1], Qiangwei Fu[2], Soizic Guihard[1], Meyling Cheok[1], Katie Miller[3], Gordon Zeng[4], Bruno Quesnel[1,5], Xavier Troussard[6], Sylvie Galiègue-Zouitina[1], and Carl Simon Shelley[2]

Abstract

Hairy cell leukemia (HCL) is characterized by underexpression of the intracellular signaling molecule RhoH. Reconstitution of RhoH expression limits HCL pathogenesis in a mouse model, indicating this could represent a new therapeutic strategy. However, while RhoH reconstitution is theoretically possible as a therapy, it is technically immensely challenging as an appropriately functional RhoH protein needs to be specifically targeted. Because of this problem, we sought to identify druggable proteins on the HCL surface that were dependent upon RhoH underexpression. One such protein was identified as CD38. Analysis of 51 HCL patients demonstrated that 18 were CD38-positive. Interrogation of the clinical record of 23 relapsed HCL patients demonstrated those that were CD38-positive had a mean time to salvage therapy 71 months shorter than patients who were CD38-negative. Knockout of the *CD38* gene in HCL cells increased apoptosis, inhibited adherence to endothelial monolayers, and compromised ability to produce tumors *in vivo*. Furthermore, an anti-CD38 antibody proved effective against pre-existing HCL tumors. Taken together, our data indicate that CD38 expression in HCL drives poor prognosis by promoting survival and heterotypic adhesion. Our data also indicate that CD38-positive HCL patients might benefit from treatments based on CD38 targeting. *Cancer Res; 75(18); 3902–11. ©2015 AACR.*

Introduction

Hairy cell leukemia (HCL) is an indolent lymphoproliferative disease characterized by pancytopenia, hepatomegaly, splenomegaly, leukocytosis, and neoplastic mononuclear cells in the peripheral blood, bone marrow, liver, and spleen (1). Complete remission rates approaching 95% can be achieved by front-line treatment with the purine nucleoside analogues pentostatin or cladribine and second-line treatments that include rituximab or vemurafenib (2, 3). However, despite these impressive statistics, a significant proportion of HCL patients either fail to respond to therapy or develop resistant disease (3). In addition, approximately 48% of patients relapse within 15 years, and as time progresses, the incidence of relapse increases (4). Because HCL usually presents in late middle-age, countries with aging populations can expect an increasing need for new treatments.

One of the diagnostic markers of HCL is abnormal expression of the gene encoding CD11c (5). Normally, this gene is transcribed only in cells of the myeloid lineage (6). However, in HCL, it is also transcribed in the neoplastic lymphocytes. This aberrant transcription is driven by constitutive binding of the proto-oncogene *JunD* to the *CD11c* gene promoter (7). Tracking back along a cascade of molecular events, we demonstrated that this activation of *JunD* is caused by constitutive signaling through the intracellular Ras pathway (7).

Signaling by members of the Ras super-family has been shown to be inhibited by high quantitative levels of RhoH (8). We have found that HCL is characterized by chronic underexpression of RhoH (9). Consequently, the low level of RhoH found in HCL likely allows members of Ras family to be active and drive disease pathogenesis. *In vitro* reconstitution of RhoH expression inhibits the aberrant expression of CD11c as well as the adhesion and transendothelial migration that are hallmarks of HCL (9). In a xenograft mouse model, RhoH reconstitution severely limits HCL pathogenesis and protects against mortality (9).

Our preclinical studies indicate that RhoH reconstitution could be a new therapy for HCL. However, the transition of this therapy from the laboratory bench to the hospital bedside is technically extremely difficult. First, it requires a recombinant protein to be introduced inside hairy cells. Second, it requires this protein to be specifically targeted only to hairy cells. Third, it requires the protein to be functionally and appropriately active when inside the cells. Because of these challenges, a protein was sought that was dependent upon RhoH underexpression but produced on the cell surface and so easily targeted.

[1]Institut National de la Santé et de la Recherche Medicale UMR-S1172, Centre Jean-Pierre Aubert, Institut pour la Recherche sur le Cancer de Lille and Université de Lille, Lille, France. [2]Kabara Cancer Research Institute, Gundersen Medical Foundation, La Crosse, Wisconsin. [3]Department of Biology, Saint Mary's University of Minnesota, Winona, Minnesota. [4]Department of Pathology, Gundersen Health System, La Crosse, Wisconsin. [5]Service des Maladies du Sang, Hôpital Huriez, Centre Hospitalier Universitaire de Lille, Lille, France. [6]Laboratoire d'Hématologie, Centre Hospitalier Universitaire de Caen, Caen, France.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Corresponding Authors: C.S. Shelley, Wisconsin School of Medicine and Public Health, 1 River Place Drive, La Crosse, WI 54601. Phone: 617-997-5097; Fax: 608-775-6602; E-mail: carlsimonshelley@yahoo.com; or S. Galiègue-Zouitina, UMR-S1172 INSERM, IRCL. Place de Verdun, 59045, Lille Cedex, France. Phone: 33-3-20-16-92-17; Fax: 33-3-20-16-92-29; E-mail: sylvie.zouitina@inserm.fr

doi: 10.1158/0008-5472.CAN-15-0893

In order to identify a cell-surface protein dependent upon RhoH underexpression, we utilized differential microarray analysis to compare the transcriptome of HCL reconstituted with RhoH with the transcriptome of nonreconstituted HCL. This analysis indicated that the mRNA encoding the cell-surface protein CD38 was dependent upon RhoH underexpression. Subsequently, this dependence was confirmed at the protein level. These findings led us to the hypothesis that CD38 could be involved in the pathogenesis of HCL, and its targeting might be therapeutic. In order to test this hypothesis, we performed functional analyses on HCL where the *CD38* gene had been knocked out. These studies indicated that CD38 promotes HCL survival, heterotypic adhesion, and the growth of xenografts in mice. That CD38 contributes to HCL pathogenesis was further demonstrated by the finding that CD38-positive patients relapsed dramatically sooner than patients who were CD38-negative. Analysis of 51 HCL patients by flow cytometry or immunohistochemistry demonstrated 18 were CD38-positive. Testing of the humanized anti-CD38 antibody SAR650984 in a mouse model indicates that this approximate one third of HCL patients could benefit from the development of anti-CD38 treatments.

Materials and Methods

Patient material

Immunohistochemical analysis was performed on formalin-fixed paraffin-embedded bone marrow biopsies collected from patients diagnosed with either classical HCL or chronic lymphocytic leukemia (CLL) at the Gundersen Medical Center, La Crosse, Wisconsin (10, 11). The prognostic ability of CD38 was determined by examining the clinical records of 43 patients diagnosed with classical HCL at the Centre Hospitalier Universitaire de Caen, Caen, France. Nine of these patients scored 3 points and 34 patients scored 4 points on the Royal Marsden scoring system for HCL (12). Cases where multiparameter flow cytometry showed that 30% or more of HCL cells exhibited CD38 expression were designated as being CD38-positive. First-line and salvage treatments were initiated when patients had platelet counts under 100×10^9/L, hemoglobin levels under 10 g/dL, or an absolute neutrophil count under 1×10^9/L.

Immunohistochemistry

Formalin-fixed paraffin-embedded blocks containing bone marrow biopsy specimens were serially sectioned at 4 μm and dried overnight on Colorfrost Plus microscope slides (Thermo Fisher Scientific, Inc.). Next, sample slides were deparaffinized, and one slide from each block was stained with hematoxylin and eosin Y. The remaining slides were subjected to epitope retrieval using Epitope Retrieval Solution, pH 9 (Dako North America, Inc.). Next, the slides were rocked with the Peroxidase Blocking reagent of the EnVision+ System-HRP (DAB; Dako North America, Inc.) and then with Surfact-Amps X-100 (Thermo Fisher Scientific, Inc.). One slide from each block was rocked with an IgG nonimmune rabbit antibody (Epitomics, Inc.). One slide from each block was identically incubated with the rabbit monoclonal antibody EPR4106 that recognizes human CD38 (Abcam, Inc.). Serial rocking incubations were next performed with Labeled Polymer-HRP Anti-Rabbit, Wash Buffer and DAB+ Chromogen (Dako North America, Inc.). Finally, slides were counterstained with hematoxylin and the tissue protected by glass coverslips mounted with Permount (Thermo Fisher Scientific, Inc.).

Cell culture

The hairy-cell line HC-1 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ). The hairy-cell line EH was provided by Guy B. Faguet (Veterans Administration Medical Center, Augusta, GA). Subsequently, the real identity of EH was found to be the hairy-cell line HK (13). Therefore, herein we refer to this line as EH/K. The hairy-cell line ESKOL was provided by Edward F. Srour (Indiana University School of Medicine, Indianapolis, IN). The hairy-cell lines JOK-1 and Hair-M were provided by Jørn Koch (Aarhus University Hospital, Aarhus, Denmark). Human microvascular endothelial cells (HMEC-1) were provided by Laurent Plawinski (CNRS UMS 3408 Université de Caen, France). The HCL cell lines JOK-Empty and JOK-RhoH were obtained as previously described and grown in RPMI-1640 containing 10% (v/v) heat-inactivated FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, and 150 μg/mL hygromycin B (Gibco Life Technologies; ref. 9). All other HCL cell lines were grown in this same medium lacking hygromycin B. HMEC-1 was grown in Medium 131 containing 100 units/mL penicillin, 100 μg/mL streptomycin, and microvascular growth supplement (MVGS; Gibco Life Technologies). In addition, surfaces on which HMEC-1 were grown were coated with attachment factor (Gibco Life Technologies). Activation of HMEC-1 was achieved using 100 ng/mL lipopolysaccharide (LPS; Sigma-Aldrich).

Generation of stable cell line pools

The plasmid pGL4.51[Luc2/CMV/Neo] contains the luciferase gene of *Photinus pyralis* under control of the constitutive gene promoter of *cytomegalovirus* (Promega Corp.). This plasmid was linearized with the restriction endonuclease *Sal*I and transfected into the HCL cell line JOK-1. The cell line pool JOK-Luc was subsequently selected by resistance to 1 mg/mL G418 (Sigma-Aldrich). Knockout of the *CD38* gene was engineered in the HCL cell line JOK-1 using a CompoZR Zinc Finger Nuclease (ZFN) kit (CKOZFND5725; Sigma-Aldrich). Parental JOK-1 cells were transfected with each of the two ZFN plasmids in the Kit and CD38-negative cells isolated by a BD FACSAria I cell sorter (Becton Dickinson) using an FITC-labeled mouse anti-human CD38 antibody and an isotype-matched nonimmune FITC-labeled antibody (clones IB6 and IS6-11E5.11, respectively; Miltenyi Biotec). CD38-negative cells were isolated as single clones in 96 multiwell culture plates using the autocloning module of the cell sorter. In the same way, CD38-positive clones were isolated from the bulk of transfected parental JOK-1 cells. Next, the *CD38* gene in each isolated clone was sequenced through the ZFN-targeted region (Supplementary Fig. S1). Six clones were identified that contained homozygous frameshift mutations within the *CD38* coding region (Supplementary Fig. S2). Six clones were identified that contained no mutations within this same region. All validated clones were first cultured alone, and then 10^6 cells of each were mixed together to produce the cell line pools JOK-CD38-WT and JOK-CD38-KO representing HCL where the *CD38* gene is wild-type or mutated, respectively. Expression of CD38 in each of these pools was assessed by Western blotting and flow cytometry (Supplementary Figs. S3 and S4, respectively).

Microarray analysis

The transcriptomes of JOK-RhoH and JOK-Empty were compared as previously described using Human Whole Genome

Agilent 44K 60-mer oligonucleotide microarrays and an Agilent DNA Microarray G2505B scanner (Agilent Technologies; ref. 14). Expression data were extracted by Feature Extraction Version 9.1.3.1 and then analyzed by GeneSpring Version 7.3 (Agilent Technologies).

Quantitative RT-PCR

Total RNA from cell cultures was purified and then reverse-transcribed into cDNA using Moloney murine lentivirus reverse transcriptase and random primers (Invitrogen, Life Technologies, Corp.; refs. 9, 14). Next, 100 ng of the generated cDNA was subjected to quantitative PCR using the Taqman Universal Master Mix and the CD38 Gene Expression Assay Hs01120068_m1 containing a *CD38*-specific TaqMan probe and primers (Applied Biosystems, Life Technologies). Linear regression curves constructed using serial dilutions of cDNA generated from the CD38-positive cell line HC-1 quantified *CD38* expression levels, which were then normalized against expression of *ABL* mRNA (9, 14). PCR was performed on a 7900HT Real-Time PCR System using the standard protocol of SDS 2.4 software (Applied Biosystems, Life Technologies).

Western blotting

Proteins were isolated from cell cultures using the M-PER lysis reagent (Thermo Fisher Scientific, Perbio Science). Proteins were then reduced using sample reducing agent (Life Technologies), subjected to polyacrylamide gel electrophoresis and transferred to nitrocellulose filters. Next, filters were incubated with primary antibodies directed against human CD38, β-actin, or glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Specifically, the anti-CD38 antibody used was mouse monoclonal 22/CD38 (BD Pharmingen). Anti–β-actin was mouse monoclonal AC-15 (Sigma-Aldrich). Anti-GAPDH was rabbit polyclonal FL-335 (Santa Cruz Biotechnology, Inc.). Following incubation with primary antibodies, filters were washed and incubated with appropriate anti-mouse or anti-rabbit secondary antibodies conjugated with horseradish peroxidase (HRP; Cell Signaling Technology, Inc., Ozyme). Filters were again washed and HRP visualized using the Amersham

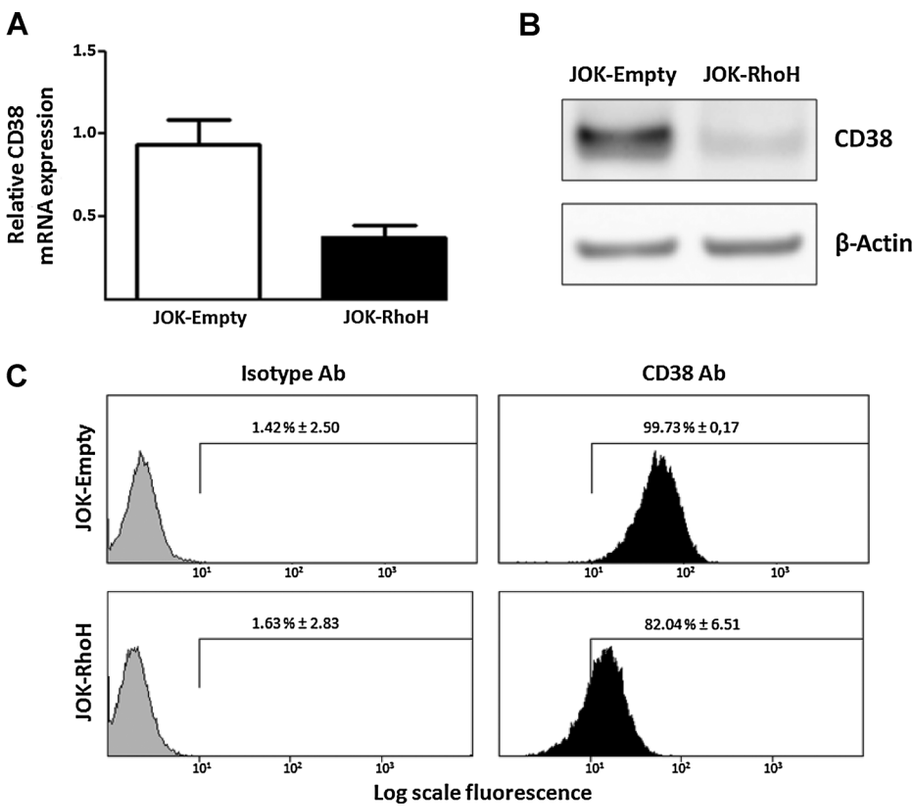

Figure 1.

Induction of RhoH in HCL downregulates CD38 expression. A, quantitative RT-PCR analysis showing that when RhoH expression is induced in JOK-1 HCL cells (JOK-RhoH), *CD38* mRNA levels are significantly reduced compared with JOK-1 HCL cells where RhoH expression is not induced (JOK-Empty; paired Student *t* test, $P = 0.0026$). Histograms represent the mean of four experiments performed in duplicate ± SD. B, Western blot analysis using an anti-CD38 mouse monoclonal antibody showing that when RhoH expression is induced in JOK-1 HCL cells (JOK-RhoH), CD38 protein levels in total protein lysates are lower than in JOK-1 where RhoH is not induced (JOK-Empty). An antibody against β-actin was used in the control analysis of lysates. C, flow cytometric analysis showing that expression of CD38 on the surface of JOK-1 cells is lower when RhoH is induced (JOK-RhoH) compared with when RhoH is not induced (JOK-Empty). Analysis was performed using an FITC-conjugated antibody that specifically binds CD38 (CD38 Ab) or an isotype-matched control antibody (Isotype Ab). A representative example of the flow patterns acquired is depicted along with the mean percentages of FITC-positive cells calculated from four experiments ± SD. After subtraction of the fluorescence intensity attributable to the control antibody, the mean fluorescence intensity attributable specifically to the CD38 antibody was 71.8 ± 14.3 SD for JOK-Empty and significantly less at 19.7 ± 9.0 SD for JOK-RhoH (paired Student *t* test, $P = 0.0006$).

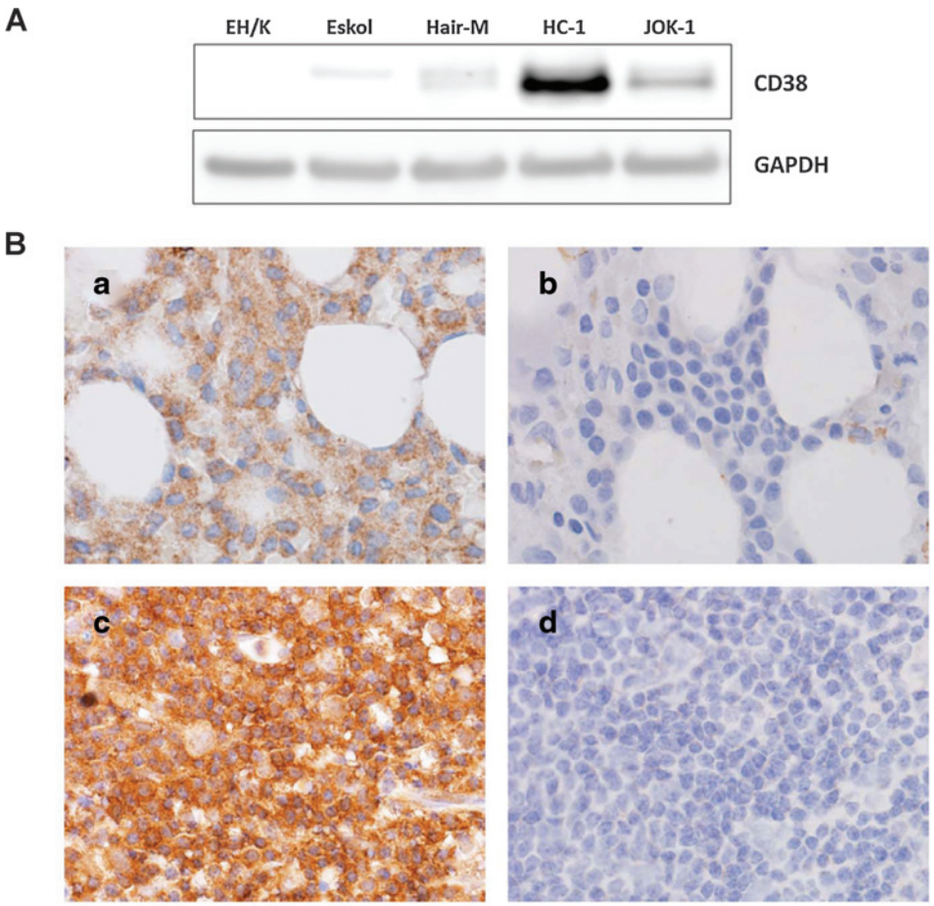

Figure 2.
CD38 is differentially expressed by HCL cell lines and patients. A, Western blot analysis of total protein lysates showing that CD38 is expressed by the HCL cell lines JOK-1, HC-1, Hair-M, and Eskol but not the HCL cell line EH/K. An antibody against GAPDH was used in the control analysis of lysates. B, immunohistochemical analysis of CD38 expression in bone marrow biopsies taken from HCL and CLL patients. Eight HCL patients were examined in this way and two found to be CD38-positive. The analysis of one of these CD38-positive patients is depicted (a). The analysis of one of the six HCL patients determined to be CD38-negative is depicted (b). As a control for the analysis of HCL patients, bone marrow biopsies taken from two CLL patients were identically examined for CD38 expression. The diagnostic pathology record of one of these CLL patients indicated that the bone marrow was CD38-positive (c). The pathology record of the second CLL patient indicated the bone marrow was CD38-negative (d). All images were taken at a magnification of ×100.

ECL Prime Western Blotting System (GE Healthcare Europe, GmbH).

Flow cytometry

Flow cytometric analysis of JOK-Empty, JOK-RhoH, JOK-CD38-WT, and JOK-CD38-KO was performed by incubating 5×10^5 cells with a FITC-conjugated version of the monoclonal antibody IB6 directed against CD38 (Miltenyi Biotec). The isotype-matched control for these experiments utilized a FITC-conjugated version of the IgG2b clone IS6-11E5.11 (Miltenyi Biotec). Following incubation with antibodies, cells were analyzed using a CyAn ADP flow cytometer equipped with Summit software 4.3 (Beckman Coulter, Inc.).

Cell-cycle assays

The percentage of cells in the S phase of the cell cycle was assessed using the Click-iT EdU Alexa Fluor 647 Flow Cytometry Assay Kit (Life Technologies). The assay consisted of labeling 5×10^5 cells with 10 μmol/L of 5-ethynyl-2′-deoxyuridine (EdU), fixation with Click-iT fixative then permeabilization with Click-iT saponin–based reagent. Next, intracellular EdU was conjugated with Alexa Fluor 647 using the Click-iT reaction cocktail. The percentage of cells that were positive for EdU-Alexa Fluor 647 was determined by flow cytometry and taken as representing the proportion of the culture in S phase.

Apoptosis assays

Cultures of JOK-CD38-WT or JOK-CD38-KO were initiated. After 72 hours, 5×10^5 cells were washed in ice-cold PBS and then incubated with FITC-conjugated Annexin V and propidium iodide (PI; Beckman Coulter, Inc.). The percentage of PI-positive cells that were also Annexin V–positive was determined by flow cytometry and taken as the proportion of the cultures that were undergoing apoptosis.

Heterotypic adhesion assays

The ability of JOK-CD38-WT or JOK-CD38-KO to adhere to HMEC-1 was assessed as previously described (9). Briefly, monolayers of HMEC-1 were produced in 96 multiwell tissue culture plates and either activated with 100 ng/mL of LPS or left untreated. JOK-CD38-WT or JOK-CD38-KO cultures were incubated with 5 μmol/L BCECF-AM (Life Technologies), washed, then 10^5 of these labeled cells set onto the monolayers. Adhesion was allowed for 1 hour at 37°C, then monolayers were washed and fluorescence intensity measured at 535 nm using a SpectraMax i3 Multi-Mode Detection Platform (Molecular Devices, LLC). All values were corrected by subtraction of the fluorescence intensity of monolayers incubated with wash buffer alone. These corrected values were then plotted against standard curves of fluorescence intensity constructed from serial dilutions of a known number of the corresponding HCL cell line labeled with BCECF-AM.

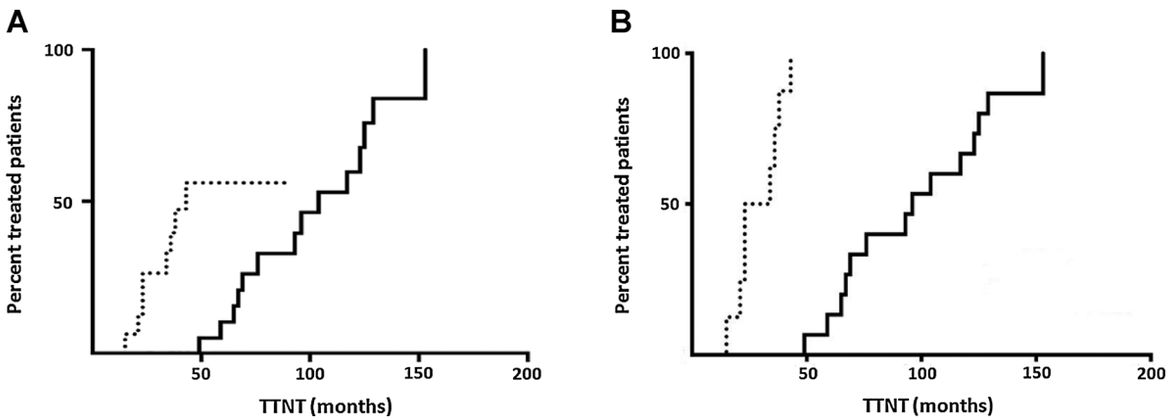

Figure 3.
CD38 is a marker of poor HCL prognosis. A, Kaplan–Meier plot showing the time between the end of first-line therapy and the beginning of salvage therapy in 43 cases of HCL. This time interval was designated as TTNT. The 16 cases that were CD38-positive and the 27 cases that were CD38-negative are plotted separately with dotted and solid lines, respectively. The mean TTNT of the CD38-positive patients was significantly shorter than that of the patients who were CD38-negative (Gehan–Breslow–Wilcoxon test, $P = 0.0023$). B, Kaplan–Meier plot showing the TTNT only of the 23 patients within the total of 43 analyzed that suffered relapse. CD38-positive and CD38-negative cases are plotted separately with dotted and solid lines, respectively. The mean TTNT of 8 CD38-positive patients was significantly shorter than that of 15 patients who were CD38-negative (Gehan–Breslow–Wilcoxon test, $P < 0.0001$). Within the CD38-positive group, 4 had first-line therapy with interferon and 4 had first-line therapy with purine analogues. Within the CD38-negative group, 6 were initially treated with interferon and 9 with purine analogues.

Mouse husbandry

Mice utilized for subcutaneous xenografts of HCL were housed in sterilized GM500 ventilated cages on a Green Line rack (Techniplast France S.A.). This housing system was kept in a barrier room accredited by the Direction Départementale de la Protection des Populations du Nord. Mice were monitored daily, and sterile water and Rat & Souris №1 Entretien diet (SDS Special Diet Services France, Argenteuil) were provided *ad libitum*. Mice utilized for intraperitoneal xenografts of HCL were housed in sterilized Super Mouse 750 Micro-Isolator ventilated cages on an RAIR Isosytem rack (Lab Products, Inc.). This housing system was kept in a barrier room accredited by AAALAC-I. Mice were monitored daily, and sterile water and Teklad Global 18% Protein Rodent Diet (Harlan Laboratories, Inc) were provided *ad libitum*.

Xenograft mouse models

The role of CD38 in HCL was assessed using 5×10^6 of the cell line pools JOK-CD38-WT or JOK-CD38-KO injected subcutaneously into male or female mice that were 6 to 8 weeks old and of the strain NOD.Cg-Prkdc^{scid} IL2rγ^{tm1Wjl}/SzJ (Jackson Laboratory). Four weeks after injection, mice were sacrificed and tumors dissected, weighed, and measured with an electronic caliper. Tumor volumes were calculated according to the formula: $(4 \times \pi \times L \times W \times T)/3$, where L is the length, W is the width, and T is the thickness. The therapeutic efficacy of targeting CD38 was assessed using 4×10^6 of the cell line pool JOK-Luc injected into the peritoneum of female mice that were 3 to 4 weeks old and of the strain Hsd:Athymic Nude-*Foxn1*^{nu} (Harlan Laboratories, Inc.). After 3 days, mice were anesthetized and injected intravenously with 150 μL of Dulbecco's Phosphate Buffered Saline containing 15 mg/mL D-Luciferin potassium salt (Regis Technologies, Inc.). Superimposed luminescence and X-ray images were acquired using a MS FX PRO In Vivo Imaging System (Bruker Corp.). The day after imaging, mice with visible tumors were injected intraperitoneally with 120 μL of PBS containing 1 mg/mL of either the humanized anti-CD38 antibody SAR650984 (Sanofi Oncology) or a nonimmune IgG1 kappa antibody purified from human myeloma plasma (Sigma-Aldrich). Two days later, the antibody injections were repeated and the following day mice were again imaged. Intraperitoneal xenograft protocols were approved by the Institutional Animal Care and Use Committee of the University of Wisconsin, La Crosse, Wisconsin. Subcutaneous xenograft protocols were approved by the Animal Care Ethical Committee, Nord-Pas-de-Calais, France.

Results

CD38 expression in HCL is dependent upon low-level RhoH

In previous studies, we determined that HCL is characterized by underexpression of the intracellular signaling molecule RhoH (9). This low-level expression likely contributes to the pathogenesis of the disease by unleashing Ras signaling that ultimately results in aberrant transcription of the *CD11c* gene and consequent extravasation of the neoplastic lymphocytes (7, 9). Reconstitution of RhoH expression ameliorated HCL pathogenesis in a xenograft mouse model (9). However, transition of this therapeutic approach to the clinic is not likely to be immanent as targeting the reconstitution of an intracellular protein is immensely challenging. Therefore, we sought a readily druggable surface protein that was dependent upon RhoH underexpression. This was achieved using the cell lines JOK-Empty and JOK-RhoH (9). These lines are derived from the HCL cell line JOK-1 and stably express either the parental vector pMEP4 or this same vector encoding RhoH. Comparison of the transcriptomes of these two derivatives by differential microarray analysis demonstrated that the mRNA encoding the surface protein CD38 is expressed in JOK-RhoH at 0.145, the level at which it is expressed in JOK-Empty ($P = 0.003$). Repression of *CD38* mRNA by RhoH reconstitution was confirmed by RT-PCR analysis (Fig. 1A). Repression of CD38 protein expression was demonstrated by Western blotting (Fig. 1B). Finally, flow cytometry demonstrated that RhoH reconstitution repressed the surface expression of CD38 (Fig. 1C).

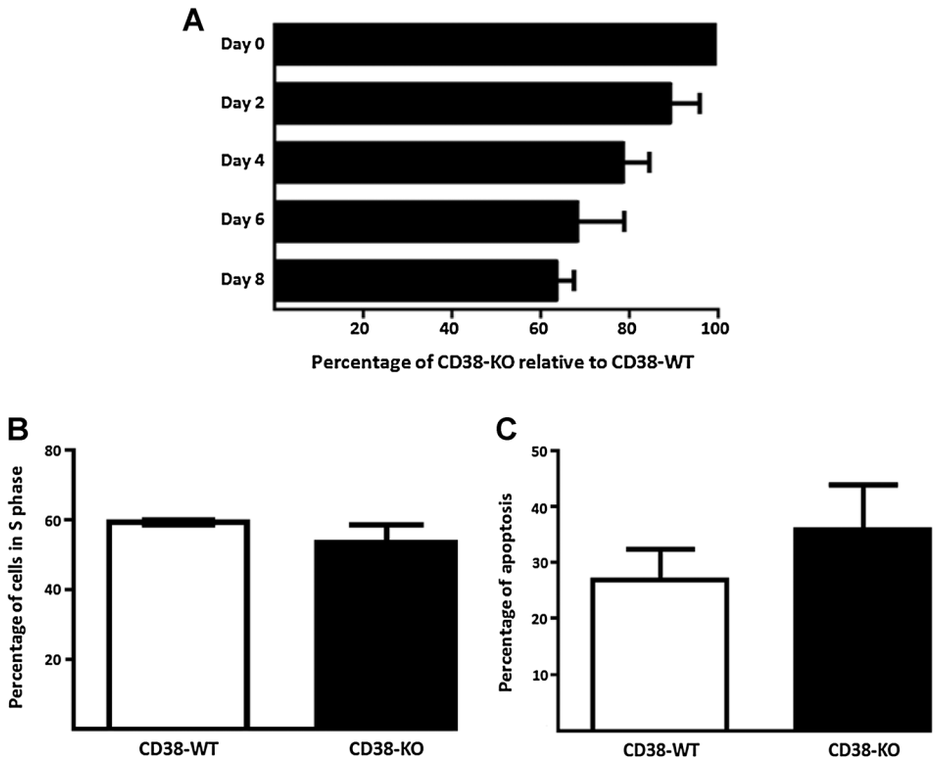

Figure 4.

CD38 promotes HCL growth. A, at day 0, cultures of JOK-CD38-KO and JOK-CD38-WT were initiated at a density of 5×10^5 cells per mL. Thereafter, cells were counted at day 2, 4, 6, and 8. At each time point, the number of JOK-CD38-WT cells (CD38-WT) was assigned a value of 100 and the number of JOK-CD38-KO cells (CD38-KO) was calculated as a percentage of this value. Histograms represent the mean ± SD of three experiments. At day 4, 6, and 8, the number of JOK-CD38-KO cells was significantly fewer than the number of JOK-CD38-WT cells (paired Student *t* test, $P = 0.0257$, 0.0342, and 0.0036, respectively). B, the rate of cell division was assessed by measuring the percentage of cells in culture that incorporated EdU and thus designated to be in the S phase of the cell cycle. Histograms represent the mean ± SD of three experiments. There is no significant difference between cultures of JOK-CD38-WT (CD38-WT) and JOK-CD38-KO (CD38-KO; paired Student *t* test, $P = 0.1571$). C, the rate of cell death was assessed by determining the percentage of cells in 72-hour cultures that expressed Annexin V and also stained with PI. These cells were designated to be undergoing apoptosis. Histograms represent the mean ± SD of four experiments. The percentage of cells undergoing apoptosis was significantly higher in cultures of JOK-CD38-KO (CD38-KO) than in cultures of JOK-CD38-WT (CD38-WT; paired Student *t* test, $P = 0.0091$).

CD38 is differentially expressed both by HCL cell lines and HCL patients

The expression of CD38 in HCL beyond JOK-1 was assessed initially by examining a range of different cell lines by Western blotting (Fig. 2A). Such analysis demonstrated that while the HCL cell lines JOK-1, HC-1, Hair-M, and Eskol are CD38-positive, the HCL line EH/K is negative. This finding of differential expression of CD38 in HCL was confirmed by analysis of HCL patients. Two of 8 HCL patients diagnosed at Gundersen Health Center in the USA exhibited CD38 expression in bone marrow biopsies (Fig. 2B). In addition, examination of the clinical records of 43 HCL patients diagnosed at the Centre Hospitalier Universitaire de Caen in France revealed 16 were CD38-positive in the bone marrow and/or peripheral blood. Taken together, the results from the USA and France indicate that the neoplastic lymphocytes of approximately one third of HCL patients exhibit CD38 expression. This proportion is consistent with what has been reported for HCL patients in Sweden (15).

CD38 expression is a marker of poor HCL prognosis

The impact of CD38 expression on clinical course was evaluated by retrospective analysis of the clinical records of the 43 patients diagnosed with classical HCL at the Centre Hospitalier Universitaire de Caen. Specifically, we investigated the time between the end of first-line therapy and the beginning of salvage therapy at first relapse. This interval was designated as the time to next treatment (TTNT). In the 43 cases, it was found that for the 27 patients who were CD38-negative, the average TTNT was 83 months, but for the 16 patients who were CD38-positive, the average TTNT was only 42 months (Fig. 3A). When we analyzed specifically the 23 of the 43 patients that relapsed, the difference in TTNT between CD38-negative and CD38-positive cases was even more dramatic. Here, the average TTNT of the 15 patients who were CD38-negative was 95 months, but only 24 months for the 8 patients who were CD38-positive (Fig. 3B).

CD38 expression promotes HCL growth by protecting against apoptosis

Clinical evidence indicates that CD38 expression in HCL correlates with poor prognosis. Next, we sought to determine if CD38 expression represents a driver or a passenger in HCL pathogenesis. This was addressed by utilizing the HCL cell line JOK-1 that is CD38 positive (Figs. 1B and 2A). Zinc Finger Nuclease technology produced pools of this line that either

contained homozygous frame-shift mutations within the *CD38* coding region or contained no mutations within this same region (Supplementary Fig. S2). These pools were designated JOK-CD38-KO and JOK-CD38-WT, respectively. Comparison of the two pools demonstrated that 8 days after the initiation of cultures with equal numbers of cells, the number of JOK-CD38-KO cells was 36% fewer than JOK-CD38-WT (Fig. 4A). The rate at which cells grow in culture represents the sum of the balance between cell division and cell death. Therefore, which of these processes accounted for the difference in growth rate of JOK-CD38-KO and JOK-CD38-WT was investigated. Incorporation of 5-ethynyl-2′-deoxyuridine demonstrated that in cultures of JOK-CD38-KO, the percentage of cells in the DNA synthesis phase of the cell cycle was not significantly different than that in cultures of JOK-CD38-WT (Fig. 4B). Consequently, CD38 expression appears not to influence HCL proliferation *in vitro*. However, in contrast, when apoptosis was assessed by cell-surface binding of Annexin V and DNA accessibility to PI, the intrinsic apoptosis rate of JOK-CD38-KO after 72 hours of culture was found to be approximately one third higher than JOK-CD38-WT (Fig. 4C). Therefore, CD38 expression appears to enhance HCL growth not by effecting an increase in proliferation, but rather by increasing cell survival.

CD38 expression promotes HCL adhesion

The adhesion of T cells and CLL B lymphocytes to endothelial cells has previously been shown to be mediated by CD38 (16, 17). Therefore, we investigated the possibility that CD38 also contributes to the adhesive properties of HCL B lymphocytes. Confluent monolayers of human microvascular endothelial cells were prepared and either activated with LPS or left untreated. The ability of JOK-CD38-KO and JOK-CD38-WT to adhere to these monolayers was then assessed (Fig. 5). This analysis demonstrated that JOK-CD38-KO was 34% less able to bind nonactivated endothelial cells than JOK-CD38-WT and 39% less able to bind activated endothelial cells.

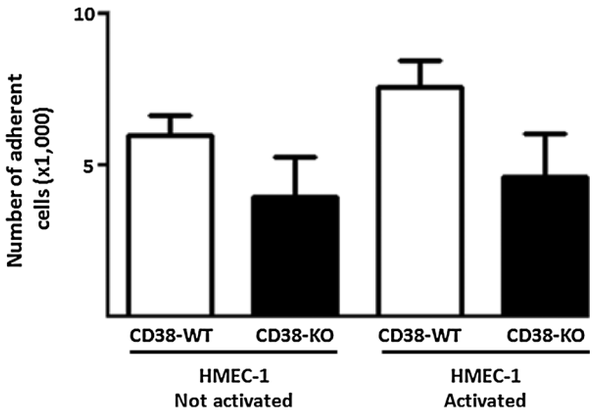

Figure 5.
CD38 promotes HCL adhesion. Confluent monolayers of human microvascular cells (HMEC-1) were prepared and either left not activated or activated with LPS. These monolayers were then incubated with JOK-CD38-WT (CD38-WT) or JOK-CD38-KO (CD38-KO) that had previously been labeled with the fluorescent marker BCECF-AM. After 1 hour, nonadherent cells were washed off and the fluorescence intensity of monolayers measured. The fluorescence of monolayers that had not been incubated with JOK cells was subtracted from this value to give a measure of the fluorescence acquired by monolayers specifically from the adherence of HCL cells. These specific fluorescence measures were then plotted on standard curves constructed from serial dilutions of a known number of the corresponding HCL cell line labeled with BCECF-AM. In this way, the number of adherent cells was calculated. Histograms represent the mean ± SD of four experiments performed at minimum in triplicate. The adherence of JOK-CD38-KO to both not activated and activated endothelial cells was significantly lower than the adherence of JOK-CD38-WT (paired Student *t* test, $P = 0.0332$ and 0.0126, respectively).

CD38 effects growth of HCL tumors *in vivo*

Analyses performed *in vitro* indicate that CD38 drives HCL survival and adhesion (Figs. 4C and 5). These results suggest CD38 expression may contribute to the pathogenesis of HCL *in vivo*. Therefore, to address this question, JOK-CD38-WT and JOK-CD38-KO were injected subcutaneously into immunodeficient mice. After 4 weeks, the resulting tumors were dissected, weighed, and their dimensions measured. This analysis demonstrated that the tumors originating from JOK-CD38-KO had volumes that were on average 40% smaller than those originating from JOK-CD38-WT (Fig. 6A). In addition, tumor weight was reduced on average by 43% (Fig. 6B). Therefore, these results support the hypothesis that CD38 influences HCL pathogenesis *in vivo*.

Targeting CD38 regresses pre-existing HCL tumors *in vivo*

Experiments performed with JOK-CD38-KO indicate that targeting CD38 expression in HCL may have therapeutic efficacy. This was tested using the parent of JOK-CD38-KO where CD38 expression remained intact. The parental line was engineered such that it constitutively produced luciferase and, therefore, could be visualized by luminescence in the presence of luciferin. This line was then injected into the peritoneum of immunodeficient mice and allowed to form tumors. These tumors were then treated with either a nonimmune control antibody or the anti-CD38 antibody SAR650984 (18). Luminescence imaging demonstrated that tumors tended to continue to grow after treatment with the control antibody but tended to be reduced by treatment with SAR650984 (Fig. 7).

Discussion

HCL is an indolent neoplasm predominantly of cells with a genetic signature related to memory B lymphocytes (1, 19). A stubborn percentage of HCL cases are either resistant to available treatments or relapse with intractable disease (3, 4). HCL usually presents in late middle age. Therefore, in regions of the world such as North America and Western Europe with a baby-boom generation reaching retirement, the absolute number of HCL patients in need of novel treatments is set to increase. Here, we report for the first time that targeting CD38 could represent one such novel therapy. We find that approximately one third of HCL patients exhibit CD38 expression, and that this expression correlates with poor prognosis. Evidence developed *in vitro* indicates that the molecular basis of this heightened pathogenesis is the ability of CD38 to effect increased lymphocyte survival and an increased ability to bind endothelium.

The natural history of CD38 expression in HCL bears a striking resemblance to that in CLL. Both are indolent neoplasms with phenotypes related to memory B cells (19–21). Approximately one third of both HCL and CLL patients are CD38-positive when the cutoff for positivity is set at 30% of the malignant clone

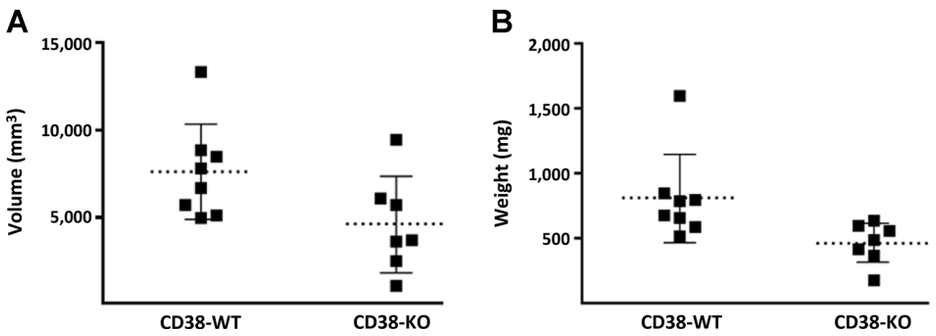

Figure 6.
CD38 promotes HCL growth *in vivo*. A, eight mice of the strain NOD.Cg-Prkdc^{scid} IL2rγ^{tm1Wjl}/SzJ were injected subcutaneously with 5×10^6 JOK-CD38-WT (CD38-WT), and seven mice of the same strain were identically injected with JOK-CD38-KO (CD38-KO). After 4 weeks, the resulting subcutaneous tumors were dissected and their volumes calculated from dimension measurements. The tumor volume in each mouse is presented as a filled square. Horizontal dotted lines show the mean tumor volumes produced by JOK-CD38-WT and JOK-CD38-KO. Vertical bars delineate the SD of these volumes. The mean volume of tumors produced by JOK-CD38-KO was significantly smaller than that of those produced by JOK-CD38-WT (unpaired Student *t* test, $P = 0.0525$). B, the weight of the same tumors where volume was calculated. The mean weight of tumors produced by JOK-CD38-KO was significantly less than that of those produced by JOK-CD38-WT (unpaired Student *t* test, $P = 0.0273$).

(22–24). In both HCL and CLL, CD38 mediates adhesion to endothelial cells (16, 17). In both HCL and CLL, CD38 protects against apoptosis (25, 26). In both HCL and CLL, CD38 positivity is a negative prognostic indicator (22, 24, 27–31).

It is now widely accepted that in CLL there are dynamic shifts of neoplastic cells between the blood stream and lymphoid tissue. In the circulation, CLL cells manifest resistance to apoptosis, but are compromised in their ability to proliferate while in lymphoid tissue they are susceptible to apoptosis or induced to proliferate (32–35). These findings indicate that the microenvironment of lymphoid tissue is necessary for CLL proliferation but not for apoptosis resistance (36). CD38 has been implicated in mediating both microenvironment-dependent CLL proliferation and microenvironment-independent survival (16, 37, 38). Extrapolating these roles of CD38 from CLL to HCL would provide an explanation for our observation that knockout of the *CD38* gene fails to influence cell division in HCL monocultures but does compromise survival.

In addition to CLL, CD38 has previously been found to be expressed in a wide range of other hematologic malignancies, including B-cell and T-cell acute lymphoblastic leukemia, multiple myeloma, B-cell non-Hodgkin's lymphoma, and acute myeloid leukemia (39–42). Targeting CD38 in xenograft models of these malignancies has been demonstrated to have therapeutic efficacy, and trials are currently under way to determine clinical utility (43–50). The results reported here indicate that HCL should be added to the list of blood cancers where anti-CD38 therapy is being evaluated. However, because monotherapy often results in the evolution of resistant disease, combining CD38 targeting with existing HCL treatments such as purine analogues and agents that target CD20 and B-Raf might prove particularly beneficial.

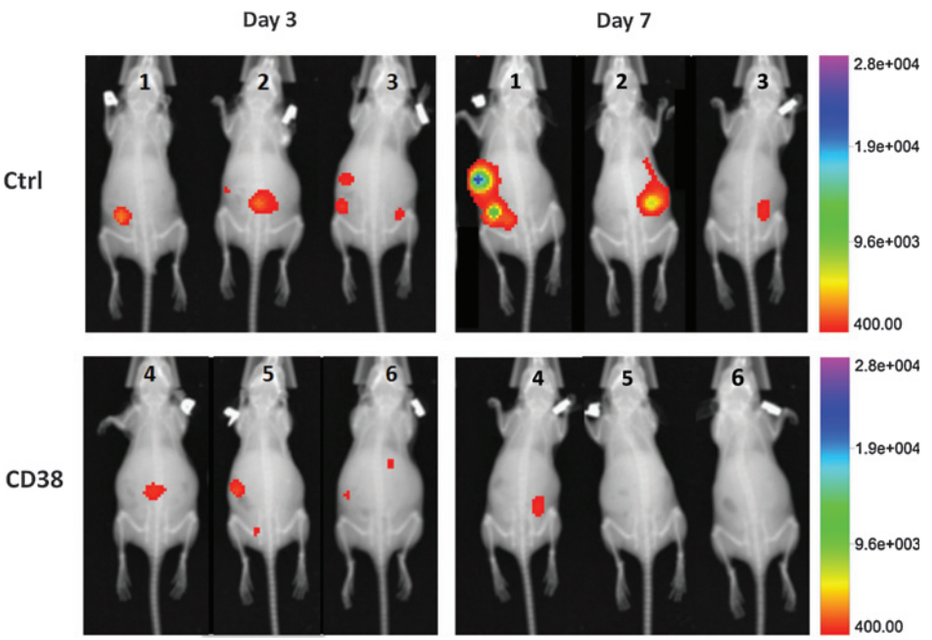

Figure 7.
Targeting CD38 treats pre-existing HCL *in vivo*. JOK-1 cells were stably transfected with the plasmid pGL4.5[Luc2/CMV/Neo] that contains the firefly *luciferase* gene under control of the constitutive gene promoter of *cytomegalovirus*. This luciferase cell line was then injected into the peritoneum of mice of the strain Hsd: Athymic Nude-*Foxn1*^{nu}. After 3 days, mice were subjected to whole body imaging following intravenous injection of D-Luciferin. Those mice where tumor was detected were intraperitoneally injected at day 4 and day 6 with the anti-CD38 antibody SAR650984 or an IgG control antibody. At day 7, mice were again imaged. Depicted are superimposed luminescence and X-ray images of three mice injected with SAR650984 (CD38) and three injected with the control antibody (Ctrl).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: N. Poret, B. Quesnel, S. Galiègue-Zouitina, C.S. Shelley
Development of methodology: N. Poret, B. Quesnel, S. Galiègue-Zouitina, C.S. Shelley
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): N. Poret, Q. Fu, S. Guihard, M. Cheok, K. Miller, G. Zeng, B. Quesnel, X. Troussard, S. Galiègue-Zouitina, C.S. Shelley
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): N. Poret, Q. Fu, M. Cheok, K. Miller, G. Zeng, B. Quesnel, X. Troussard, S. Galiègue-Zouitina, C.S. Shelley
Writing, review, and/or revision of the manuscript: N. Poret, M. Cheok, K. Miller, X. Troussard, S. Galiègue-Zouitina, C.S. Shelley
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Q. Fu, S. Guihard, B. Quesnel, X. Troussard, S. Galiègue-Zouitina, C.S. Shelley
Study supervision: S. Galiègue-Zouitina, C.S. Shelley

Acknowledgments

The authors thank Jørn Koch, Edward Srour, Guy Faguet, and Laurent Plawinski for providing cell lines; Sanofi Oncology for the kind gift of SAR650984; Nathalie Jouy for help with flow cytometry analyses; and Steven Cash for help with immunocytochemistry. They are indebted to IMPRT IFR114 for use of their Animalerie Haute-Technologie Plateforme de Ressources Expérimentales, their Beckman Coulter flow cytometer, Applied Biosystems Real-Time PCR System, and their Becton Dickinson flow cytometer and cell sorter. They thank Amy Cooper for animal husbandry, Elisabeth Bertrand and Pauline Peyrouze for help with animal experiments, and Brooklynn Scholze, Pat Fisher, Linda Wright, and Julie Seiler for searches of pathology files. In addition, they thank Martin Figeac, Frédéric Leprêtre, Sabine Quief, and Céline Villenet for microarray analyses and Maud Collyn d'Hooghe for extended IRCL support of NP. Finally, they are indebted to the Mary Westlund Family Foundation for purchase of a Sakura Histo-Tek SL auto-staining apparatus and to Betty Zane Kabara for purchase of a Bruker MS FX PRO In Vivo Imaging System and an Olympus BX43 microscope with image capture capability.

Grant Support

This study was supported by Institut pour la Recherche sur le Cancer de Lille, Comité du Nord and Comité du Pas de Calais of Ligue Nationale Francaise Contre le Cancer and Université de Lille, Institut National de la Santé et de la Recherche Médicale (INSERM), SIRIC OncoLille grant INCa-DGOS-INSERM 6041aa. Sanofi Oncology, the Kabara Cancer Research Institute, and the Hairy Cell Leukemia Research Foundation supported testing of SAR650984.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received April 6, 2015; revised June 1, 2015; accepted June 22, 2015; published online September 15, 2015.

References

1. Bouroncle BA, Wiseman BK, Doan CA. Leukemic reticuloendotheliosis. Blood 1958;13:609–30.
2. Robak T. Current treatment options in hairy cell leukemia and hairy cell leukemia variant. Cancer Treat Rev 2006;32:365–76.
3. Sári E, Nagy ZG, Baghy K, Rajnai H, Bödör C, Csomor J, et al. Treatment of refractory hairy cell leukemia with BRAF-inhibitor: lessons to be learnt. Pathol Oncol Res 2014;20:973–80.
4. Else M, Dearden CE, Matutes E, Garcia-Talavera J, Rohatiner AZS, Johnson SAN, et al. Long-term follow-up of 233 patients with hairy cell leukaemia, treated initially with pentostatin or cladribine, at a median of 16 years from diagnosis. Br J Haematol 2009;145:733–40.
5. Schwarting R, Stein H, Wang CY. The monoclonal antibodies αS-HCL (αLeu-14) and αS-HCL3 (αLeu-M5) allow the diagnosis of hairy cell leukemia. Blood 1985;65:974–83.
6. Shelley CS, Böttinger EP, Arnaout MA. Transcriptional regulation of β2 integrins. Leukocyte adhesion: basic and clinical aspects. Amsterdam: Elsevier Science Publishers B.V.; 1992. p. 337–51.
7. Nicolaou F, Teodoridis JM, Park H, Georgakis A, Farokhzad OC, Böttinger EP, et al. *CD11c* gene expression in hairy-cell leukemia is dependent upon activation of the proto-oncogenes *ras* and junD. Blood 2003;101:4033–41.
8. Li X, Bu X, Lu B, Avraham H, Flavell RA, Lim B. The hematopoietic-specific GTP-binding protein RhoH is GTPase deficient and modulates activities of other Rho GTPases by an inhibitory function. Mol Cell Biol 2002;22:1158–71.
9. Galiègue-Zouitina S, Delestré L, Dupont C, Troussard X, Shelley CS. Underexpression of RhoH in hairy cell leukemia. Cancer Res 2008;68:4531–40.
10. Fu Q, Cash SE, Andersen JJ, Kennedy CR, Oldenburg DG, Zander VB, et al. CD43 in the nucleus and cytoplasm of lung cancer is a potential therapeutic target. Int J Cancer 2013;132:1761–70.
11. Fu Q, Cash SE, Andersen JJ, Kennedy CR, Madadi AR, Raghavendra M, et al. Intracellular patterns of sialophorin expression define a new molecular classification of breast cancer and represent new targets for therapy. Br J Cancer 2014;110:146–55.
12. Matutes E, Morilla R, Owusu-Ankomah K, Houliham A, Meeus P, Catovsky D. The immunophenotype of hairy cell leukemia (HCL). Proposal for a scoring system to distinguish HCL from B-cell disorders with hairy or villous lymphocytes. Leuk Lymphoma 1994;14 Suppl 1:57–61.
13. Drexler HG, Dirks WG, Matsuo Y, MacLeod RAF. False leukemia-lymphoma cell lines: an update on over 500 cell lines. Leukemia 2003;17:416–26.
14. Delestré L, Berthon C, Quesnel B, Figeac M, Kerckaert J-P, Galiègue-Zouitina S, et al. Repression of the *RHOH* gene by JunD. Biochem J 2011;437:75–88.
15. Juliusson G, Lenkei R, Liliemark J. Flow cytometry of blood and bone marrow cells from patients with hairy cell leukemia: phenotype of hairy cells and lymphocyte subsets after treatment with 2-chlorodeoxyadenosine. Blood 1994;83:3672–81.
16. Deaglio S, Mallone R, Baj G, Arnulfo A, Surico N, Dianzani U, et al. CD38/CD31, a receptor/ligand system ruling adhesion and signaling in human leukocytes. Chem Immunol 2000;75:99–120.
17. Patten PE, Buggins AG, Richards J, Wotherspoon A, Salisbury J, Mufti GJ, et al. CD38 expression in chronic lymphocytic leukemia is regulated by the tumor microenvironment. Blood 2008;111:5173–81.
18. Deckert J, Wetzel MC, Bartle LM, Skaletskaya A, Goldmacher VS, Vallée F, et al. SAR650984, a novel humanized CD38-targeting antibody, demonstrates potent antitumor activity in models of multiple myeloma and other CD38+ hematologic malignancies. Clin Cancer Res 2014;20:4574–83.
19. Basso K, Liso A, Tiacci E, Benedetti R, Pulsoni A, Foa R, et al. Gene expression profiling of hairy cell leukemia reveals a phenotype related to memory B cells with altered expression of chemokine and adhesion receptors. J Exp Med 2004;199:59–68.
20. Klein U, Tu Y, Stolovitzky GA, Mattioli M, Cattoretti G, Husson H, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J Exp Med 2001;194:1625–38.
21. Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 2001;194:1639–48.
22. Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94:1840–7.
23. Deaglio S, Vaisitti T, Aydin S, Ferrero E, Malavasi F. In-tandem insight from basic science combined with clinical research: CD38 as both marker and key component of the pathogenetic network underlying chronic lymphocytic leukemia. Blood 2006;108:1135–44.
24. Matrai Z. CD38 as a prognostic marker in CLL. Hematology 2005;10:39–46.
25. Deaglio S, Vaisitti T, Bergui L, Bonello L, Horenstein AL, Tamagnone L, et al. CD38 and CD100 lead a network of surface receptors relaying positive signals for B-CLL growth and survival. Blood 2005;105:3042–50.

26. Pepper C, Ward R, Lin TT, Brennan P, Starczynski J, Musson M, et al. Highly purified CD38^{+} and CD38^{-} sub-clones derived from the same chronic lymphocytic leukemia patient have distinct gene expression signatures despite their monoclonal origin. Leukemia 2007;21:687–96.
27. Del Poeta G, Maurillo L, Venditti A, Buccisano F, Epiceno AM, Capelli G, et al. Clinical significance of CD38 expression in chronic lymphocytic leukemia. Blood 2001;98:2633–9.
28. Ibrahim S, Keating M, Do KA, O'Brien S, Huh YO, Jilani I, et al. CD38 expression as an important prognostic factor in B-cell chronic lymphocytic leukemia. Blood 2001;98:181–6.
29. Jelinek DF, Tschumper RC, Geyer SM, Bone ND, Dewald GW, Hanson CA, et al. Analysis of clonal B-cell CD38 and immunoglobulin variable region sequence status in relation to clinical outcome for B-chronic lymphocytic leukaemia. Br J Haematol 2001;115:854–61.
30. Morabito F, Mangiola M, Oliva B, Stelitano C, Callea V, Deaglio S, et al. Peripheral blood CD38 expression predicts survival in B-cell chronic lymphocytic leukemia. Leuk Res 2001;25:927–32.
31. Dürig J, Naschar M, Schmücker U, Renzing-Köhler K, Hölter T, Hüttmann A, et al. CD38 expression is an important prognostic marker in chronic lymphocytic leukaemia. Leukemia 2002;16:30–5.
32. Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. Blood 2009;114:3367–75.
33. Calissano C, Damle RN, Hayes G, Murphy EJ, Hellerstein MK, Moreno C, et al. In vivo intraclonal and interclonal kinetic heterogeneity in B-cell chronic lymphocytic leukemia. Blood 2009;114:4832–42.
34. Zenz T, Mertens D, Küppers R, Döhner H, Stilgenbauer S. From pathogenesis to treatment of chronic lymphocytic leukaemia. Nat Rev Cancer 2010;10:37–50.
35. Damle RN, Calissano C, Chiorazzi N. Chronic lymphocytic leukaemia: a disease of activated monoclonal B cells. Best Pract Res Clin Haematol 2010;23:33–45.
36. Deaglio S, Malavasi F. Chronic lymphocytic leukemia microenvironment: shifting the balance from apoptosis to proliferation. Haematologica 2009;94:752–6.
37. Malavasi F, Deaglio S, Damle R, Cutrona G, Ferrarini M, Chiorazzi N. CD38 and chronic lymphocytic leukemia: a decade later. Blood 2011;118:3470–8.
38. Deaglio S, Vaisitti T, Zucchetto A, Gattei V, Malavasi F. CD38 as a molecular compass guiding topographical decisions of chronic lymphocytic leukemia cells. Semin Cancer Biol 2010;20:416–23.
39. Keyhani A, Huh YO, Jendiroba D, Pagliaro L, Cortez J, Pierce S, et al. Increased CD38 expression is associated with favorable prognosis in adult acute leukemia. Leuk Res 2000;24:153–9.
40. Konopleva M, Rissling I, Andreeff M. CD38 in hematopoietic malignancies. Chem Immunol 2000;75:189–206.
41. Leo R, Boeker M, Peest D, Hein R, Bartl R, Gessner JE, et al. Multiparameter analyses of normal and malignant human plasma cells: CD38++, CD56+, CD54+, cIg+ is the common phenotype of myeloma cells. Ann Hematol 1992;64:132–9.
42. Schuurman H-J, Huppes W, Verdonck LF, van Baarlen J, van Unnik JAM. Immunophenotyping of non-Hodgkin's lymphoma. Correlation with relapse-free survival. Am J Pathol 1988;131:102–11.
43. Stevenson FK, Bell AJ, Cusack R, Hamblin TJ, Slade CJ, Spellerberg MB, et al. Preliminary studies for an immunotherapeutic approach to the treatment of human myeloma using chimeric anti-CD38 antibody. Blood 1991;77:1071–9.
44. Goldmacher VS, Bourret LA, Levine BA, Rasmussen RA, Pourshadi M, Lambert JM, et al. Anti-CD38-blocked ricin: an immunotoxin for the treatment of multiple myeloma. Blood 1994;84:3017–25.
45. de Weers M, Tai YT, van der Veer MS, Bakker JM, Vink T, Jacobs DC, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. J Immunol 2011;186:1840–8.
46. Chillemi A, Zaccarello G, Quarona V, Ferracin M, Ghimenti C, Massaia M, et al. Anti-CD38 antibody therapy: windows of opportunity yielded by the functional characteristics of the target molecule. Mol Med 2013;19:99–108.
47. Ellis JH, Barber KA, Tutt A, Hale C, Lewis AP, Glennie MJ, et al. Engineered anti-CD38 monoclonal antibodies for immunotherapy of multiple myeloma. J Immunol 1995;155:925–37.
48. Mehta K, Ocanas L, Malavasi F, Marks JW, Rosenblum MG. retinoic acid-induced CD38 antigen as a target for immunotoxin-mediated killing of leukemia cells. Mol Cancer Ther 2004;3:345–52.
49. Flavell DJ, Boehm DA, Emery L, Noss A, Ramsay A, Flavell SU. Therapy of human B-cell lymphoma bearing SCID mice is more effective with anti-CD19- and anti-CD38-saporin immunotoxins used in combination than with either immunotoxin used alone. Int J Cancer 1995;62:337–44.
50. Mihara K, Yanagihara K, Takigahira M, Kitanaka A, Imai C, Bhattacharyya J, et al. Synergistic persistent effect of T-cell immunotherapy with anti-CD19 or anti-CD38 chimeric receptor in conjunction with rituximab on B-cell non-Hodgkin lymphoma. Br J Heamatol 2010;151:37–46.

Cancer Research

Correction: CD38 in Hairy Cell Leukemia Is a Marker of Poor Prognosis and a New Target for Therapy

In this article (Cancer Res 2015;75:3902–11), which appeared in the September 15, 2015, issue of *Cancer Research* (1), there was erroneous duplication of one of the panels of Fig. 7. Specifically, the panel depicting mouse 4 treated with the CD38 antibody SAR650984 and imaged 7 days after injection of the cell line pool JOK-Luc was erroneously duplicated. The erroneous duplicate was presented as mouse 3 treated with an IgG control antibody and imaged 7 days after injection of JOK-Luc. Fig. 7 has now been corrected to show the correct 7-day image of mouse 3 treated with the IgG control.

This change makes more apparent the original scientific conclusion that CD38 is a new target for the therapy of hairy cell leukemia.

The corrected figure appears below. The legend remains the same as originally published. The authors regret the error.

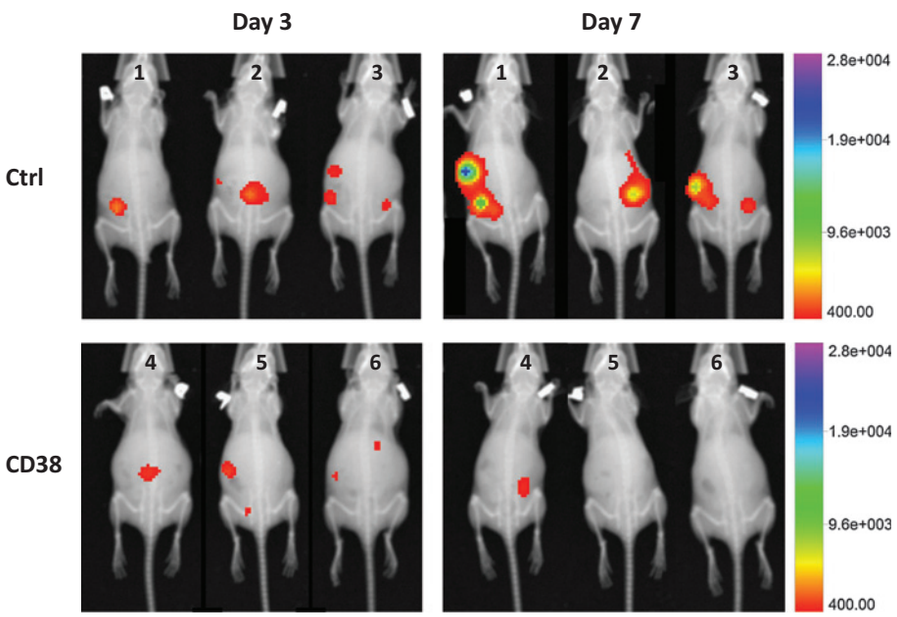

Figure 7.

Reference

1. Poret N, Fu Q, Guihard S, Cheok M, Miller K, Zeng G, et al. CD38 in hairy cell leukemia is a marker of poor prognosis and a new target for therapy. Cancer Res 2015;75:3902–11.

Published online December 1, 2015.
doi: 10.1158/0008-5472.CAN-15-2913