SAR650984, a novel humanized CD38-targeting antibody, demonstrates potent anti-tumor activity in models of multiple myeloma and other CD38+ hematologic malignancies

Authors: Jutta Deckert¹, Marie-Cécile Wetzel², Laura M. Bartle¹, Anna Skaletskaya¹, Victor S. Goldmacher¹, François Vallée³, Qing Zhou-Liu⁴, Paul Ferrari⁴, Stéphanie Pouzieux³, Charlotte Lahoute²,§, Charles Dumontet⁵, Adriana Plesa⁵, Marielle Chiron², Pascale Lejeune²,*, Thomas Chittenden¹, Peter U. Park¹,#, Véronique Blanc²

Affiliations:
¹ Research&Development, ImmunoGen Inc., Waltham, MA, USA
² Oncology Business Division, Sanofi R&D, Vitry-sur-Seine, France
³ SCP LGCR/Structure Design & Informatics/Structural Biology, Sanofi R&D, Vitry-sur-Seine, France
⁴ Biorealization/Operations and Protein Tools, Sanofi R&D, Vitry-sur-Seine, France
⁵ Hematology Laboratory, Hospices Civils de Lyon, Pierre Bénite, France and CRCL, INSERM 1052-CNRS 5286, Lyon, France


Keywords: CD38, Antibody, Multiple Myeloma

Corresponding Author: Jutta Deckert, ImmunoGen, Inc., 830 Winter Street, Waltham, MA 02451, Phone 781-895-0600, jutta.deckert@immunogen.com

Conflict-of-interest statement: M.C.W., F.V., Q.Z.L., P.F., S.P., C.L., M.C., P.L. and V.B. are or were employees of sanofi, J.D., L.M.B., A.S., V.S.G., T.C. and P.U.P. are or were employees of ImmunoGen, Inc.

5,122 words, 6 Figures
Abstract

**Purpose:** The CD38 cell surface antigen is expressed in diverse hematologic malignancies including multiple myeloma (MM), B-cell non-Hodgkin lymphoma (NHL), B-cell chronic lymphocytic leukemia (B-CLL), B-cell acute lymphoblastic leukemia (ALL) and T-cell ALL. Here we assessed the anti-tumor activity of the anti-CD38 antibody SAR650984.

**Experimental Design:** Activity of SAR650984 was examined on lymphoma, leukemia and MM cell lines, primary MM samples and MM xenograft models in immunodeficient mice.

**Results:** We identified a humanized anti-CD38 antibody with strong pro-apoptotic activity independent of cross-linking agents, and potent effector functions including complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP), equivalent in vitro to rituximab in CD20+ and CD38+ models. This unique antibody, termed SAR650984, inhibited the ADP-ribosyl cyclase activity of CD38, likely through an allosteric antagonism as suggested by 3D structure analysis of the complex. In vivo, SAR650984 was active in diverse NHL, ALL and MM CD38+ tumor xenograft models. SAR650984 demonstrated single agent activity comparable to rituximab or cyclophosphamide in Daudi or SU-DHL-8 lymphoma xenograft models with induction of the pro-apoptotic marker cleaved capase 7. In addition, SAR650984 had more potent anti-tumor activity than bortezomib in NCI-H929 and Molp-8 MM xenograft studies.
Consistent with its mode of action, SAR650984 demonstrated potent pro-apoptotic activity against CD38+ human primary MM cells.

**Conclusion:** These results validate CD38 as a therapeutic target and support the current evaluation of this unique CD38-targeting functional antibody in Phase I clinical trials in patients with CD38+ B-cell malignancies.

**Translational Relevance**

The CD38 antigen is a promising target for an antibody-based therapeutic as it is expressed in a number of hematologic malignancies including multiple myeloma, B-cell non-Hodgkin lymphoma, B-cell chronic lymphocytic leukemia (B-CLL), B-cell acute lymphoblastic leukemia (ALL) and T-cell ALL. Based on the success of rituximab in treating CD20+ lymphoma, targeting CD38 with a functional antibody is a promising therapeutic approach for these diverse indications. SAR650984, a novel anti-CD38 antibody described here, induces a unique combination of effects on cancer cells including CDC, ADCC, ADCP and apoptosis, and demonstrates potent in vitro and in vivo activity against cells derived from these malignancies. Consequently, clinical trials with this antibody in patients with CD38+ hematologic malignancies are on-going (NCT01084252, NCT01749969), with initial signs of promising efficacy.
Introduction

Rituximab, a chimeric anti-CD20 antibody, dramatically improved the treatment outcome of both indolent and aggressive non-Hodgkin lymphoma (NHL) through its use as a single agent or in combination with chemotherapy (1, 2). While rituximab ultimately depletes B cells through binding to CD20, its mechanism of action is attributed to a combination of direct apoptosis induction and activation of immune effector mechanisms, including antibody-dependent cell-mediated cytotoxicity (ADCC) and phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) (3). Despite the success of rituximab, incomplete treatment responses and emergence of resistance represent important limitations (1). In an attempt to overcome these limitations, a plethora of second generation anti-CD20 antibodies have been introduced into clinical development, most notably ofatumumab and obinutuzumab which have gained regulatory approval in B-cell chronic lymphocytic leukemia (CLL) (4). CD20 expression is confined to a B-cell subset, restricting the utility of these agents to patients with CLL and B-cell NHL. Therefore, identification of new targets with a broader expression spectrum and potential for antibody mechanisms similar to rituximab could yield novel antibody therapeutics for a wider range of hematologic malignancies.

The cell surface molecule CD38 represents such an opportunity since it is expressed in multiple hematologic malignancies derived from both lymphoid and myeloid lineages, including multiple myeloma (MM), a CD20 negative B-cell malignancy (5). CD38 is a 45 kDa type II transmembrane glycoprotein that has been described as both a receptor and a multifunctional enzyme involved in the production of nucleotide-metabolites (6-8). It has been proposed to associate with cell surface proteins in lipid rafts, regulate
cytoplasmic Ca$^{2+}$ flux and mediate signal transduction in lymphoid and myeloid cells (9-12). During hematopoiesis CD38 is expressed on CD34+ stem cells and lineage-committed progenitors of lymphoid, erythroid and myeloid cells, while pluripotent early stem cells are defined as CD34+CD38-. CD38 expression is absent during the intermediate stages of lymphocyte maturation, then reappears during the final stages of maturation and continues through the plasma cell stage (13-16).

CD38 expression is an important prognostic factor in B-CLL that identifies patients with aggressive disease and poor survival (17-19). It has been proposed that CD38 plays a role in the proliferation and survival of CLL cells (20). High CD38 expression has been reported on plasma cells as well as their malignant counterparts, MM cells, leading to its widespread use as a surface marker, combined with CD138, to enumerate malignant myeloma cells in patient bone marrow (5, 21). Overall, CD38 expression in hematologic malignancies and its correlation with disease progression makes CD38 an attractive target for antibody-based therapeutics. Several anti-human CD38 antibodies differing in their functional properties against various malignant cells and cell lines have been described in the literature (10, 22-27). A recently developed antibody, daratumumab, is currently undergoing clinical trials in MM patients and reported to have a manageable safety profile and early signs of efficacy (28-30). Another anti-CD38 antibody, MOR202, is being evaluated in a phase 1/2a trial in patients with relapsed/refractory MM (31, 32).

Here we describe the generation of a new humanized anti-CD38 antibody, SAR650984, selected for its multiple mechanisms of action. SAR650984 demonstrated potent pro-apoptotic activity in the absence of cross-linking agents, elicited immune effector mechanisms such as CDC, ADCC and ADCP against CD38+ malignant cells and in
addition strongly inhibited CD38 enzymatic activity. This unique combination of activities translated into strong in vivo efficacy of SAR650984 against diverse CD38+ tumor xenograft models.

Materials and methods

Cell culture

Ramos, Raji, Daudi, and Namalwa lymphoma cells were obtained from American Type Culture Collection (ATCC). All other cell lines were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 2 mM glutamine and 1% penicillin-streptomycin (Life Technologies) at 37°C in a humidified 5% CO₂ incubator.

Antibody generation

CD38-binding murine monoclonal antibodies were generated by standard hybridoma technology following immunizations with murine 300-19 cells transfected to express the full-length human CD38 antigen. Antibodies were chimerized (ch) as huIgG1-kappa and humanized (hu) by variable domain resurfacing (33). Chimeric antibodies chHB7 and chOKT10 were produced based on sequences derived from their respective murine hybridomas (ATCC HB-136, CRL-8022). Ab005 (a surrogate antibody for daratumumab) and huAT13/5 were produced based on published sequences (Genbank: ADS96869 and ADS96865, corresponding to sequences 17 and 12 from US7829673 for Ab005, CAA03366 and CAA03368 for huAT13/5). IB4 and SUN-4B7 were a gift from...
F. Malavasi, University of Turin, Italy. Rituximab, cyclophosphamide and bortezomib were obtained from commercial sources.

**Apoptosis induction**

Cells were incubated at 2 x 10^5 cells/mL in complete medium (RPMI-1640, 10% FBS, 2mM L-glutamine) with 1.5 μg/mL (10 nM) of indicated antibodies for 20 hours at 37°C with 5% CO₂. Cells were stained with AnnexinV-FITC in accordance with the manufacturer’s instructions (Life Technologies). Samples were analyzed by flow cytometry on a FACSCalibur flow cytometer with CellQuest Pro (v5.2) for acquisition control and data analysis (both BD Biosciences).

**Binding assay**

Antibodies were incubated with 5 x 10⁴ cells/assay in RPMI-1640 with 2% normal goat serum and 0.1% sodium azide (both Sigma-Aldrich) for 3 hours on ice followed by incubation with FITC-conjugated goat-anti-mouse or anti-human IgG antibody (Jackson ImmunoResearch) for 1 hour on ice. Samples were analyzed on a FACSCalibur flow cytometer as above. Sigmoidal dose-response curves were generated and EC₅₀ values calculated using GraphPad Prism (GraphPad Software, San Diego).

**Expression analysis**

Cells were incubated in PBS with 0.1% sodium azide and 0.5% BSA (Sigma-Aldrich) at 2 x 10⁵ cells/assay with 5 μg/mL anti-CD38-PE or 16 μg/mL anti-CD20-PE (BD Biosciences) for 2 hours on ice. Samples were washed, fixed and acquired in conjunction with QuantiBRITE beads (BD Biosciences) by flow cytometry according to
the manufacturer’s instructions. Values for antibodies bound per cell (ABC) were extrapolated from the standard curve.

**ADCC, ADCP and CDC assays**

Antibody-dependent cell mediated cytotoxicity (ADCC) activity with isolated natural killer (NK) cells and $5 \times 10^4$ target cells at an effector:target (E:T) ratio of 3:1 was measured by lactate dehydrogenase (LDH) release (Cytotoxicity Detection Kit, Roche) after 4 hour incubation using standard protocols (34). The degree of specific lysis was calculated as follows: % lysis = (experimental release - spontaneous release)/(maximum release - spontaneous release) x 100.

Monocyte-derived macrophages were prepared by isolating monocytes from peripheral blood using CD14+ beads and culturing them in the presence of 50 ng/mL hGM-CSF (both Miltenyi Biotec) for 7 days. Antibody-dependent cellular phagocytosis (ADCP) was evaluated using $2 \times 10^5$ Ramos target cells labeled with PKH26 (Sigma-Aldrich), then incubated with antibodies and isolated human monocyte-derived macrophages in round-bottom 96-well plates at $37^\circ$C for 3 hours at an E:T ratio of 3:1. Subsequently, samples were stained with anti-CD11b-Alexa Fluor®-488 (AF, Life Technologies) to identify macrophages, washed and fixed with 4% paraformaldehyde. Cell-associated fluorescence of PKH26 (FL2, Target cells) and AF (FL1, Macrophages) was measured by flow cytometry using a FC500 cytometer. Dual-labeled cells were considered as phagocytic events and the degree of phagocytosis was determined as: %phagocytosis = 100 x [# of FL2+FL1+ events/ (# of FL2+FL1+ events + # of FL2+FL1- events)].
In complement-dependent cytotoxicity (CDC) assays, antibodies were incubated with 5 x 10^4 target cells/well in the presence of 5% human complement (Sigma-Aldrich) for 2 hours as previously described (35). Viability of remaining cells was determined by AlamarBlue assays (Life Technologies).

**Primary MM samples**

Bone marrow aspirates from MM patients were obtained from Hospital Edouard Herriot, Lyon, France after written informed consent under protocols approved by an institutional review board. Mononuclear cells were prepared by standard Ficoll-Paque centrifugation. Cells were incubated at 10^6 cells/sample with 100 µg/mL SAR650984 for 18 hours at 37°C in culture media containing 50% autologous serum (all patient samples contained > 18 mg/mL of IgG; see Supplementary Table S1). Samples were stained with anti-CD38-APC (HB7, BD Biosciences) and anti-CD138-PC5 (BA38, Beckman Coulter) antibodies to identify MM cells and the degree of apoptosis was measured by Annexin-V-FITC staining. Samples were analyzed by flow cytometry on a FACSCalibur or a FACS Canto II (both BD Biosciences).

**Enzymatic activity assay**

A recombinant soluble human CD38-Fc fusion protein was produced, and enzymatic activity assays carried out as described in the Supplementary Data section (36).

**3D structure analysis**

Crystals of the non-glycosylated soluble human CD38 antigen (extracellular domain R45-I300) and the SAR650984 Fab fragment were obtained and analyzed as described in the Supplementary Data section. High resolution data for crystals up to 1.53 Å were...
collected at beamline ID-29 at the European Synchrotron Radiation Facility (ESRF, Grenoble, France).

**In vivo xenograft studies**

Female CB.17 severe combined immunodeficient (SCID) mice (Charles River Laboratories) were inoculated subcutaneously (s.c.) with $1 \times 10^7$ cells. Treatment was initiated as indicated and tumor volumes were measured twice weekly for the duration of the study. %T/C was calculated as the median tumor volume of each treated (T) group divided by the median tumor volume of the vehicle control (C) group. Activity was assessed according to NCI standards (%T/C $\leq 42\% =$ active, %T/C value $\leq 12\% =$ highly active).

For survival studies, SCID mice were inoculated intravenously (i.v.) with $5 \times 10^6$ cells/mouse through lateral tail vein injection. Animals were sacrificed when hind leg paralysis was present or body weight decreased by $>20\%$ of pre-treatment weight. Kaplan Meyer analysis was performed for survival studies and % increase of lifespan (%ILS) was calculated as %ILS = 100x (Median survival of treated group - median survival of control group)/ median survival of control group. In accordance with NCI standards %ILS $> 25\%$ was considered active. Induction of cleaved caspase 7 in tumors was evaluated by immunoblot as described in the Supplementary Data section.
Results

Antibody generation and selection

We generated a large panel of murine monoclonal antibodies that specifically bound to human CD38 (huCD38). In order to identify antibodies with intrinsic anti-cancer cell activity, antibodies were screened for their ability to induce apoptosis in lymphoma cells in the absence of any cross-linking agents. One of the antibodies identified had notably strong pro-apoptotic activity, inducing 35% Annexin V-positive Daudi cells compared to 6% Annexin V-positive cells in the absence of antibody (Fig. 1A). In contrast, other anti-CD38 antibodies generated in our screen, such as clones 7 and 23, did not induce a significant increase in Annexin V staining of cells. Previously generated anti-CD38 antibodies huAT13/5, chOKT10, IB4, and SUN-4B7 also resulted at best in a minor (14% or less) increase in the percentage of Annexin V-positive Daudi cells. This murine lead clone was therefore chosen for further analysis based on its unique pro-apoptotic activity and humanized by variable domain resurfacing. The chimeric and humanized versions of the lead clone retained specific, high-affinity binding to human CD38 with an apparent Kd of 0.2 nM (Fig. 1B). Its humanized IgG1 version was designated as SAR650984.

Functional activity of the humanized anti-CD38 antibody SAR650984

Superior pro-apoptotic activity by SAR650984 over rituximab in the absence of cross-linking agents was seen in Raji, Daudi and Ramos lymphoma cells, which express CD20 and CD38 at similar levels (Fig. 2A and B). In addition SAR650984 induced
apoptosis in CD38+CD20- SU-DHL-8 DLBCL cells, Molp-8 MM cells and DND-41 T-ALL cells (Fig. 2A).

Immune effector functions mediated by rituximab were reported to contribute to its clinical activity. We therefore compared immune effector activities of SAR650984 in CD38+CD20+ cell lines with those of rituximab. SAR650984 induced strong complement-dependent cytotoxicity (CDC) in the presence of human serum as well as strong antibody-dependent cell-mediated cytotoxicity (ADCC) via human NK cells in Raji and Daudi cells, with similar activity as rituximab (Fig. 2C and D). In addition, SAR650984 mediated antibody-dependent cell-mediated phagocytosis (ADCP) with human macrophages against Ramos cells to a similar extent as rituximab at 10 μg/mL (Fig. 2E).

**SAR650984 activity against a panel of diverse cell lines**

CD38 is expressed on a wider range of malignant cell types than CD20, providing the potential for an expanded utility of a therapeutic anti-CD38 antibody (5). We therefore characterized a larger panel of cell lines for their CD38 expression level and SAR650984 in vitro activity.

CD38 levels were determined by quantitative flow cytometry and ranged from 790,000 to 59,000 antibodies bound per cell (ABC) in the 15 cell lines tested representing B-cell NHL, MM, B-CLL, T-cell acute lymphoblastic leukemia (ALL) and B-cell ALL (Fig. 3A). Most notably the 3 MM cell lines evaluated ranked among the six highest expressors with 790,000 to 233,000 ABC. SAR650984 induced apoptosis in 8 of 15 evaluated cell lines with up to 90% AnnexinV-positive cells and EC$_{50}$ values from 2-16 ng/mL (Fig. 3B).
Strong CDC activity was seen in 7 of 15 cell lines evaluated with up to 90% maximum lysis and EC$_{50}$ values varying widely from 8-230 ng/mL (Fig. 3C). SAR650984 mediated potent ADCC against all 15 cell lines evaluated with maximum lysis of 30% to 90% of target cells and EC$_{50}$ values of the dose-response ranged from approximately 0.2-8 ng/mL (Figure 3D). SAR650984 mediated ADCP with 60% phagocytosed Ramos cells, compared to 25% in untreated samples, with an EC$_{50}$ value of 5 ng/mL (Fig. 3E). While ADCC activity was seen against all CD38+ cell lines tested regardless of CD38 expression level, apoptosis induction and CDC activity was most often seen against cell lines with the highest CD38 levels (Fig. 3A).

Binding of SAR650984 to normal human peripheral blood cells was assessed by flow cytometry. SAR650984 specifically bound to monocytes and T cells (Supplementary Fig. S1A and B) with the same affinity seen on tumor cells. We observed low levels of CD38 expression in normal human blood cells with 3,000 to 14,000 ABC, much lower than levels of CD20 expression on B cells (Supplementary Fig. S1C). SAR650984 treatment did not deplete peripheral blood cells cultured in vitro (Supplementary Fig. S1D), whereas rituximab resulted in B-cell depletion and alemtuzumab in both B- and T-cell depletion.

**Apoptosis induction in primary MM patient cells**

The ability of SAR650984 to induce apoptosis without the addition of external crosslinking agent was also tested in primary cells isolated from bone marrow aspirates of 7 MM patients. SAR650984 noticeably increased the percentages of AnnexinV positive cells over background levels in all MM samples tested with a mean increase of 25% AnnexinV-positive cells (Fig. 3F).
Enzymatic activity

Inhibition of the adenosine diphosphate (ADP) ribosyl-cyclase enzymatic activity of human CD38 by SAR650984 and other anti-CD38 antibodies was evaluated in vitro. SAR650984 inhibited the enzymatic function of 5 nM recombinant CD38 almost completely at concentrations of 200 and 20 nM with partial inhibition at 2 nM concentration (Figure 4A). A human IgG1 isotype control antibody had no measurable inhibitory effect in these experiments. Other anti-CD38 antibodies tested, Ab005 (a surrogate antibody for daratumumab), chHB7, huAT13/5 and chOKT10, resulted in much lower or no inhibitory effect (Fig. 4B).

3D structure of the huCD38/SAR650984-Fab complex

Mapping of the epitope and identification of the paratope was achieved by determining the crystal structure of soluble human CD38 (huCD38) in complex with the Fab fragment from SAR650984 at 1.53 Å resolution (PDB ID code: 4CMH, Fig. 4C). Residues that are part of the huCD38 epitope bound by SAR650984-Fab are highlighted in Figure 4D and notably include a continuous segment from Met110 to Cys119. Mutations introduced to silence the four glycosylation sites of CD38 are not part of the huCD38/SAR650984-Fab interface.

A close-up view of the epitope shows that the interface between huCD38 and SAR650984-Fab involves only part of the residues in the CDR loops (Fig. 4E). Heavy chain CDR Loop H3 protrudes out of the structure of SAR650984-Fab and is particularly critical for complex stabilization while Loop H1, H2, L1, L2 and L3 are located at the periphery of the interface. Four salt-bridges form the interactions between H1 and H2 with Arg194 and Lys111 of huCD38 (Supplementary Fig. S2).
Fab induces local conformational changes in the huCD38 structure. The most significant conformational change affects the segment Gln115-Thr116-Val117 of huCD38 that is shifted by ~2.5Å towards the light chain, creating enough space to accommodate the H3 loop of SAR650984-Fab (Fig. 4E). However, the configuration of key residues involved in the ADP-ribosyl cyclase activity of CD38 is maintained and the catalytic site remains accessible (Fig. 4F), suggesting SAR650984 is likely an allosteric antagonist.

**In vivo activity against lymphoma and leukemia models**

We evaluated the in vivo efficacy of SAR650984 in comparison to established lymphoma treatments such as rituximab and chemotherapy. SAR650984 showed similar anti-tumor activity as rituximab in a disseminated xenograft model established from CD20+ CD38+ Daudi lymphoma cells (Fig. 5A). Treatment with 40 mg/kg SAR650984 or rituximab twice weekly for 3 weeks markedly increased survival with a median increase in lifespan (%ILS) of 68% for SAR650984 and 52% for rituximab as compared to untreated control animals. SAR650984 was also highly efficacious in a disseminated NALM-6 B-ALL xenograft model, with a median %ILS of >200% at doses of ≥ 2.5 mg/kg (Fig. 5B). In a subcutaneous xenograft model derived from SU-DHL-8, a diffuse large B-cell lymphoma cell line, SAR650984 was active at all doses tested with T/C values of 8%, 14%, 14% or 26% at the 40, 20, 10 or 5 mg/kg dose, respectively (Fig. 5C). No significant weight loss was observed as a result of SAR650984 treatment in any of these studies. Cyclophosphamide (CPA), an established lymphoma treatment component, was inactive at the 56 mg/kg dose level, moderately active at 145 and 90 mg/kg (T/C values of 3% or 17%, respectively), and was not tolerated at a higher dose of 234 mg/kg. SU-DHL-8 tumors excised after a single SAR650984 treatment were
analyzed for the levels of cleaved caspase 7, a marker for apoptotic pathway activation, in comparison to untreated tumors. SAR650984 induced a 64% or 169% increase in cleaved caspase 7 levels after 14 or 24 hours, respectively (Fig. 5D).

**In vivo activity against multiple myeloma models**

Next, we examined the utility of SAR650984 in subcutaneous xenograft models derived from CD20-CD38+ MM cell lines Molp-8 and NCI-H929 in comparison with bortezomib, an established MM treatment. In a study with palpable Molp-8 tumors SAR650984 was well tolerated and active at 40, 25, and 15 mg/kg administered twice weekly for three weeks with T/C values of 8%, 10%, and 12%, respectively (Fig. 6A). Bortezomib treatment at 0.7 or 0.4 mg/kg administered twice weekly for two weeks was inactive, while higher doses of 1.2 or 1.9 mg/kg were toxic.

Similarly, in a study with established NCI-H929 tumors, SAR650984 treatment was well tolerated and highly active at 40, 20, and 10 mg/kg x6 with 10/10 tumor-free survivors and at 5 mg/kg x6 with 9/10 tumor-free survivors at study end (Fig. 6B). Bortezomib administered twice weekly for two weeks was highly active at 0.6 mg/kg (T/C 7.8%; 4/10 tumor-free survivors), and at 1 mg/kg (10/10 tumor-free survivors). A higher bortezomib dose of 1.6 mg/kg was toxic, while a dose of 0.4 mg/kg was inactive.

**Discussion**

The introduction of therapeutic monoclonal anti-CD20 antibodies, such as rituximab, ofatumumab and very recently obinutuzumab, are improving outcomes for patients with
B-cell malignancies, although unmet medical need remains. It would be desirable to develop antibody therapeutics for other hematologic diseases, in particular MM, that could also combine high efficacy with low systemic toxicity. Our goal was to build upon the success story of CD20 antibody therapy by developing a novel multi-functional antibody directed against a different target with expanded utility in a range of hematologic malignancies. Here, we describe the generation and characterization of SAR650984, a CD38-targeting antibody with a unique combination of anti-tumor activities.

The ability of SAR650984 to induce apoptosis in vitro was superior to rituximab in B-cell lymphoma cell lines with similar CD38 and CD20 expression, with potent activity even in the absence of cross-linking agents. This type of enhanced pro-apoptotic activity has been described for type II anti-CD20 antibodies such as tositumomab or obinutuzumab (37). In addition, SAR650984 induced apoptosis in MM and T-ALL cell lines, which are typically CD20-, as well as a CD20- DLBCL cell line. Similarly to rituximab, SAR650984 mediated ADCC and ADCP effector activities with potent ADCC activity seen against cell lines derived from diverse CD38+ malignancies. Unlike type II anti-CD20 antibodies, SAR650984 also induced CDC in lymphoma cell lines with activity comparable to rituximab. SAR650984 demonstrated potent in vivo anti-tumor activities similar to rituximab in both disseminated and subcutaneous CD20+CD38+ lymphoma models. Against DLBCL SU-DHL-8 tumors SAR650984 activity compared favorably to CPA, a component of the standard treatment regimen CHOP, whose in vivo activity was limited by toxicity. In this model SAR650984 also induced cleaved caspase 7, a marker of apoptotic cell death. Notably, SAR650984 was active against Molp-8 and NCI-H929 MM
xenograft tumors, while bortezomib, an agent commonly used in MM treatment, was only active against NCI-H929 tumors.

Several anti-CD38 antibodies with various functional properties have been identified previously. While some of these can mediate in vitro killing of CD38+ cell lines via ADCC and/or CDC effector function, their direct impact on cell proliferation or apoptosis remains unclear (10, 22-24). For example, the agonistic antibody IB4 was reported to prevent apoptosis or induce proliferation in some contexts (10, 38), and induce apoptosis in another (9). The more recently developed antibody daratumumab has been shown to mediate ADCC as well as ADCP but to induce apoptosis only in the presence of stromal cells or cross-linking agents (28, 39). It was selected based on its CDC activity, which may be linked to its unique epitope. Another recent addition, MOR202, primarily possesses ADCC activity with CDC and ADCP also being observed with no reported intrinsic pro-apoptotic activity (31, 32).

Analysis of the 3D structure of SAR650984-Fab with human CD38 revealed that SAR650984 binds to a discontinuous epitope which includes amino acids located opposite to the catalytic site of CD38. While significant conformational changes are observed in CD38 upon binding by SAR650984, the overall configuration of key residues involved in the CD38 enzymatic activity and access to the catalytic site appear conserved (40-42). Nevertheless, SAR650984 strongly inhibits CD38 enzymatic activity suggesting that SAR650984 is an allosteric rather than an orthosteric antagonist of CD38. This is different from HB7, an antibody that does not compete with SAR650984 (Supplementary Fig. S3), binds at a different location in the C-terminal portion of the CD38 extracellular domain (Supplementary Fig. S4) and does not inhibit CD38.
enzymatic activity (Fig. 4B). The SAR650984 binding mode appears unique as other anti-CD38 antibodies tested here or described elsewhere show no or much less potent inhibition of the enzymatic activity.

CD38 enzymatic activities produce nucleotide-metabolites including cADPR, which induce Ca2+ mobilization and signaling (7, 8, 43). However, the physiological role of CD38 is still unknown, as functional studies have mainly used cell lines ectopically overexpressing CD38 and anti-CD38 antibodies, which are non-physiological ligands (44, 45). Therefore, the exact interplay between enzymatic activity, receptor function and consequences on signaling pathways and cellular response remains to be further documented. As a consequence, the physiological impact of SAR650984 inhibition of CD38 enzymatic activity remains to be deciphered and how the functional differences observed between various anti-CD38 antibodies affect their therapeutic utility requires further investigation. The contribution of distinct mechanisms of action of SAR650984 to clinical efficacy may also differ depending on the type of hematologic malignancy, tumor biology and tumor microenvironment.

Use of chemotherapy and autologous stem cell transplantation and the availability of new therapeutic agents including proteasome, kinase, and histone deacetylase inhibitors, as well as immune-modulatory drugs have improved survival in myeloma patients (46, 47). However, the majority of patients will eventually relapse underscoring the need for additional therapeutic options. Moreover, many of these small molecule agents have significant toxicities, such as neuropathy, that limit their dosing and their potential for use in combination therapy. Drawing on the example of rituximab, with its favorable safety profile and success in combination therapy for lymphoma, a functional
CD38 antibody could offer the prospect of a highly effective therapy in MM that might also be used readily in combination with other agents to improve clinical outcomes. Based on the results described here, SAR650984 has shown potent in vitro and in vivo activity against diverse CD38+ lymphoma, leukemia as well as MM-derived cell line models and can induce apoptosis in primary MM cells. Therefore, it presents an exciting opportunity for a novel antibody therapeutic in CD38+ hematologic malignancies and is currently in clinical testing in MM patients with early signs of efficacy (48).

Acknowledgments

We would like to thank Daniel Tavares, Michele Mayo, Hélène Bastien, Josiane Le Parc, François Michoux, Anne Pommeret, Dominique Quarteronet, Florence Attenot and Catherine Geslin for technical support, Ti Cai for critical reading of the manuscript and Walter Blättler, Thierry Hercend, Michel Streuli and John Lambert for guidance and helpful discussions.

Authorship

Contribution: J.D. performed experiments and wrote the manuscript, M.-C.W., F.V., Q.Z.-L., and P.F. performed experiments and contributed to manuscript writing, A.P., L.B., A.S., S.P., C.L., and M.C. performed experiments and analyzed data, P.L. supervised studies, C.D. supervised studies and edited the manuscript, V.S.G., T.C., P.U.P., and V.B. designed the research and contributed to manuscript writing.
References


34. Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, et al. High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. J Biol Chem. 2001;276:6591-604.


**Figure Legends**

**Figure 1. Identification of the lead anti-CD38 antibody.** (A) Induction of apoptosis by indicated antibodies in Daudi cells measured by AnnexinV-FITC staining. (B) Binding of the humanized anti-CD38 antibody SAR650984 and its chimeric variant to Daudi cells by flow cytometry.

**Figure 2. In vitro activity of SAR650984 and rituximab.** (A) Induction of apoptosis by SAR650984 or rituximab in various cell lines. (B) CD20 and CD38 expression in lymphoma cells by flow cytometry. (C) CDC activity against Raji (left) or Daudi (right) cells. (D) ADCC activity against Raji (left) or Daudi (right) target cells (E) ADCP activity against Ramos target cells.

**Figure 3. In vitro activity of SAR650984 on CD38+ cells.** (A) CD38 expression by quantitative flow cytometry. Representative dose-response curve examples for (B) Apoptosis induction, (C) CDC activity, (D) ADCC activity and (E) ADCP activity of SAR650984. (F) Apoptosis induction by SAR650984 in CD38+CD138+ primary MM cells.

**Figure 4. CD38 enzymatic activity and 3D-structure of the huCD38/SAR650984-Fab complex.** (A) Inhibition of CD38 enzymatic activity by SAR650984 measured in vitro by the production of cyclic GDPRibose (cGDPR) from NGD+. (B) Inhibition of CD38 enzymatic activity at 90 minutes in the presence of 20 nM of various anti-CD38 antibodies. (C) Overall structure of the complex in surface and ribbon representation with SAR650984-Fab heavy chains (yellow), light chains (green) and human CD38 (gray). (D) Amino-acid sequence of the R45-I300 huCD38 extracellular domain with...
SAR650984-Fab epitope residues in blue, mutations introduced to silence the four glycosylation sites of CD38 in light gray, CD38 active site residues in red. Epitope residues were defined as residues which contain atoms within 4Å from any atom of the SAR650984-Fab CDRs. (E) Detailed view of the epitope highlighting the contributions of loops H3 (yellow) and L1, L2 and L3 (green). (F) Overlay of CD38/SAR650984–Fab on free CD38 (PBD 2EF1, red), CD38 in complex with GTP (PBD 3DZH, magenta) and with NAD (PBD 2I65, cyan). Essential residues of CD38 involved in the enzymatic activity are shown in stick representation.

**Figure 5. In vivo efficacy of SAR650984 in lymphoma and leukemia xenograft models.** SCID mice (n=10 per group) bearing xenografts were treated with i.p. administration of SAR650984 or reference compounds as indicated, (A) disseminated Daudi xenografts, (B) disseminated Nalm-6 xenografts, (C) subcutaneous SU-DHL-8 lymphoma xenografts treated with SAR650984 or cyclophosphamide (CPA). (D) Induction of cleaved caspase 7 after a single dose of 40 mg/kg SAR650984 in SU-DHL-8 xenograft in SCID mice evaluated by immunoblot.

**Figure 6. In vivo efficacy in MM xenograft models.** SCID mice bearing subcutaneous xenografts of (A) Molp-8 or (B) NCI-H929 cells treated as indicated with SAR650984 or bortezomib.
Figure 1

A

% Annexin V positive cells

B

Mean Fluorescence Intensity

- chimeric Ab
- humanized Ab (SAR850984)
Figure 2

A

B

C

D

E

% Annexin V positive

no Ab
SAR650984
rituximab

Raji
Daudi
Ramos
SU-DHL-6
D14

% Viability

Ab concentration (ng/mL)

% Lysis

Ab concentration (ng/mL)

% ADCC

Ab concentration (ng/mL)
Figure 3
Figure 5

A

% Survival

Days (post inoculation)

PBS
SAR650984
rituximab

B

% Survival

Days (post inoculation)

PBS
SAR650984
40mg/kg
10mg/kg
2.5mg/kg

C

Median Tumor Volume (mm³)

Days (post inoculation)

Control
SAR650984 40mg/kg
SAR650984 10mg/kg
SAR650984 20mg/kg
SAR650984 5mg/kg
CPA 145mg/kg
CPA 90mg/kg
CPA 56mg/kg

D

% Cleared Caspase 7 induction

Days

4 h
14 h
24 h
Figure 6

A

Median Tumor Volume (mm$^3$)

- PBS
- SAR650984 40 mg/kg
- SAR650984 25 mg/kg
- SAR650984 15 mg/kg
- bortezomib 0.7 mg/kg
- bortezomib 0.4 mg/kg

B

Median Tumor Volume (mm$^3$)

- PBS
- SAR650984 40 mg/kg
- SAR650984 20 mg/kg
- SAR650984 10 mg/kg
- SAR650984 5 mg/kg
- bortezomib 1.0 mg/kg
- bortezomib 0.6 mg/kg
- bortezomib 0.4 mg/kg
SAR650984, a novel humanized CD38-targeting antibody, demonstrates potent anti-tumor activity in models of multiple myeloma and other CD38+ hematologic malignancies

Jutta Deckert, Marie-Cecile Wetzel, Laura M Bartle, et al.

*Clin Cancer Res* Published OnlineFirst July 1, 2014.

**Updated version**
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-14-0695

**Supplementary Material**
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2014/07/14/1078-0432.CCR-14-0695.DC1

**Author Manuscript**
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.