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Daratumumab, a Novel Therapeutic Human CD38 Monoclonal Antibody, Induces Killing of Multiple Myeloma and Other Hematological Tumors

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CD38, a type II transmembrane glycoprotein highly expressed in hematological malignancies including multiple myeloma (MM), represents a promising target for mAb-based immunotherapy. In this study, we describe the cytotoxic mechanisms of action of daratumumab, a novel, high-affinity, therapeutic human mAb against a unique CD38 epitope. Daratumumab induced potent Ab-dependent cellular cytotoxicity in CD38-expressing lymphoma- and MM-derived cell lines as well as in patient MM cells, both with autologous and allogeneic effector cells. Daratumumab stood out from other CD38 mAbs in its strong ability to induce complement-dependent cytotoxicity in patient MM cells. Importantly, daratumumab-induced Ab-dependent cellular cytotoxicity and complement-dependent cytotoxicity were not affected by the presence of bone marrow stromal cells, indicating that daratumumab can effectively kill MM tumor cells in a tumor-preserving bone marrow microenvironment. In vivo, daratumumab was highly active and interrupted xenograft tumor growth at low dosing. Collectively, our results show the versatility of daratumumab to effectively kill CD38-expressing tumor cells, including patient MM cells, via diverse cytotoxic mechanisms. These findings support clinical development of daratumumab for the treatment of CD38-positive MM tumors.

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Multiple myeloma (MM) is a malignant disorder of the B cell lineage, characterized by neoplastic monoclonal expansion of plasma cells in the bone marrow resulting in destruction of adjacent bone tissue. MM remains an incurable disease (median overall survival, 4.4–7.1 y) despite the recent availability of new agents that, especially when used in combination regimens, have dramatically improved initial response rates (1–3). Therefore, new approaches that induce long-term tumor regression and improve disease outcome are needed and urgently sought for.

Targeted immunotherapy with mAbs has become critical for the successful treatment of many forms of cancer. This is exemplified by rituximab, a chimeric CD20 Ab, which has revolutionized the treatment of several B cell malignancies such as follicular lymphoma (4, 5). B cells, however, lose CD20 expression upon terminal differentiation into plasma cells, and rituximab consequently has conveyed very limited benefit to the treatment of MM (6–8).

In cancer immunotherapy, effective therapeutic Abs are typically thought to require both cytostatic and cytotoxic abilities (9). This can be optimally achieved if Ab binding to the target directly interrupts signaling and, in addition, activates potent cytotoxic immune effector functions such as Ab-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Thus, cell surface-expressed molecules that 1) play an important role in cell growth or survival and that 2) are highly overexpressed on malignant cells represent attractive targets. Both of these requirements are in place for CD38 (10–12).

CD38 is a 46-kDa type II transmembrane glycoprotein with a short 20-aa N-terminal cytoplasmic tail and a long 256-aa extracellular domain (13). Functions ascribed to CD38 include receptor-mediated adhesion and signaling events, as well as important bifunctional ectoenzymatic activities that contribute to intracellular calcium mobilization (10). Under normal conditions, CD38 is expressed at relatively low levels on lymphoid and myeloid cells and in some tissues of nonhematopoietic origin (14). The relatively high expression of CD38 on all malignant cells in MM (12) in combination with its role in cell signaling suggest CD38 as a potential therapeutic Ab target for the treatment of MM.

In the past, several Abs to human CD38 have been generated that induce killing of neoplastic B cell lines (15–17). Two CD38 mAbs are currently in clinical development: a humanized mAb (SAR650984) (18) and a human mAb (daratumumab; current work). Daratumumab represents an Ab with unique cytotoxic activities. Significantly, daratumumab was found to be able to effectively kill MM tumor cells that were freshly isolated from patients by ADCC and CDC. Additionally, daratumumab was active at low concentrations in a SCID mouse xenograft tumor model. Dar-

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Abbreviations used in this article: ADCC, Ab-dependent cellular cytotoxicity; BMSC, bone marrow stromal cell; calcine-AM, calcine acetoxymethyl ester; CDC, complement-dependent cytotoxicity; KLH, keyhole limpet hemocyanin; MM, multiple myeloma; PI, propidium iodide.

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atramumab is currently in a phase I/II safety and dose finding study for the treatment of MM.

Materials and Methods

Cells

NIH 3T3 cells were transfected to express human CD38 (NIH-3T3-CD38) and used for immunizations. Briefly, NIH 3T3 cells (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) were transfected with pCitrusCD38 vector (provided by Prof. M. Glennie, Tenovus Research Laboratory, Southampton General Hospital, Southampton, U.K.) using lipofectamine (Invitrogen, Breda, The Netherlands) according to the manufacturer’s instructions. By using limiting dilution, a high-expressing cell line was selected. Human CD38 transfected CHO cells (CHO-CD38) were provided by Prof. M. Glennie. Daudi cells were obtained from the American Type Culture Collection (LGC Standards, Teddington, U.K.).

Daudi-luc cells and UM-9-luc cells were generated as described previously (19–21). Ramos cells were provided by Prof. J. Golab (Department of Immunology, Center of Bioststructure Research, The Medical University of Warsaw, Warsaw, Poland). Cells were cultured in RPMI 1640 medium supplemented with 1% (v/v) sodium pyruvate (BioWhittaker, Walkersville, MD), 1% (v/v) l-glutamine, 1% (v/v) penicillin and streptomycin, and 10% (v/v) fetal calf serum (FCS) (HyClone, Logan, UT).

Primary CD138-positive MM cells (Manassas, VA), or by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). Primary CD138-positive MM cells from patients were obtained after Institutional Review Board-approved (Dana-Farber Cancer Institute) informed consent using positive selection with CD138 microbeads (Miltenyi Biotec, Auburn, CA). Informed consent was obtained from all patients in accordance with the Declaration of Helsinki. Residual CD138-negative bone marrow mononuclear cells were obtained from all patients in accordance with the Declaration of Helsinki. Informed consent and after approval by the Medical Ethical Committee (University Medical Center, Utrecht, The Netherlands). Relapsed patients had been treated extensively with a median of three treatment regimens that included induction therapy with anthracyclines followed by autologous stem cell transplantation, thalidomide, and bortezomib. In these experiments, negative selection was performed to obtain MM tumor cells using Abs directed against CD33 mAb (BD Pharmingen), CD11b mAb (BD Pharmingen), CD52 mAb, CD45RA mAb, CD45 mAb, anti-glycophenin A mAb, CD3 mAb, CD14 mAb (all from Dianova), and magnetic beads coated with human anti-mouse IgG (Invitrogen). After this negative selection procedure, the percentage of tumor cells varied between 75 and 92%, as determined by assessing the fraction of CD138-positive cells in flow cytometry.

Construction of hemagglutinin-CD38 expression vector pEE13.4HACD38

The extracellular domain of human CD38 was amplified from plasmid pCitrusCD38 (obtained from Prof. M. Glennie). By PCR, sequences encoding an efficient signal peptide, an N-terminal hemagglutinin (HA)-tag, suitable restriction sites and an ideal Kozak sequence (GCCGCCACC) for optimal expression were introduced. The resulting PCR fragment was cloned into expression vector pEE13.4 (Lonza Biologies, Slough, U.K.) and after confirmation of the complete coding sequence, this construct was named pEE13.4HACD38.

Transient expression in HEK-293F cells

Freestyle 293F (HEK-293F) cells were obtained from Invitrogen and transfected with pEE13.4HACD38 and with the construct carrying the mutation S274F, according to the manufacturer’s protocol using 293fectin (Invitrogen), producing recombinant HA-CD38 (extracellular domain of human CD38 with an N-terminal HA-tag). Culture supernatants of transfected cells were used in ELISA for CD38 mAb binding studies.

Purification of HA-CD38

HA-CD38 was purified from the supernatant using a CD38-Ab column, by coupling Sepharose 4B (Amersham Biosciences, Uppsala, Sweden) to CD38 mAb AT13/5. For immunization, HA-CD38 was coupled to murine keyhole limpet hemocyanin (KLH) using the Iject Imnom g EDC conjugation kit (PierceThermo Fisher Scientific; Perbio Science, Etten-Leur, The Netherlands) according to the manufacturer’s instructions.

Ab generation

CD38 Abs were generated by immunization of HuAb-mice (Medarex, Milpitas, CA) with purified HA-CD38 recombinant protein alone or alternating with NIH-3T3-CD38 cells. After isolation of mouse splenocytes and lymph node cells and fusion, the resulting hybridomas were tested for binding to CHO-CD38 cells. All HuAbs described in this article were of the IgG1, κ subclass. From this panel of 42 Abs, daratumumab was selected. In all experiments, anti-KLH, a human IgG1 Ab directed at KLH, was used as negative control (25).

Flow cytometry

Experiments were performed on ice. CHO-CD38 and Daudi cells were incubated with serial dilutions of CD38 mAb or isotype control. After washing and incubation with FITC-conjugated rabbit anti-human IgG (Dako, Heverlee, Belgium), cell-associated fluorescence was determined using a FACS-Calibur with CellQuest Pro software (BD Biosciences, Erembodegem-Aals, Belgium).

MM tumor cells were incubated with mouse anti-human CD138 Ab (Dako) and serial dilutions of FITC-conjugated CD38 mAbs. After washing and incubation with PE-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA), cell-associated fluorescence was again assessed by flow cytometry, and analysis was performed after gating for CD38 expression.

ADCC

Lysis of plasma cell leukemia cells and MM tumor cells by ADCC was measured in a [51Cr]-release assay as described (26). In experiments with plasma cell leukemia cells, an E:T ratio of 40:1 was used. In experiments with MM tumor cells, an E:T ratio of 100:1 was used.

In some cases, MM cell lines and CD38-purified patient MM cells were subjected to calcein acetoxymethyl ester (calcein-AM) release ADCC assays as described elsewhere (23). The percentage of specific lysis was determined in the similar formula as in [51Cr]-release ADCC assay.

Complement-dependent cytotoxicity

Lysis of Daudi cells and MM cells via CDC was measured by use of flow cytometry (FACS-Calibur) after measurement of the percentage of propidium iodide (PI)-positive cells, as described previously (27, 28). Pooled human serum (10%) was used as source of complement. MM tumor cells were first stained for CD38 and goat anti-mouse IgG FITC as conjugate (Jackson ImmunoResearch Laboratories). MM cell lysis was determined after calculating the viable cells within the CD38-positive cell population. For data shown in Table I, GraphPad software was used to generate dose-response curves, and top curve values were used to determine maximal lysis, which was reached between 0.3 and 1.2 µg/ml.

In a separate set of experiments, MM cells were subjected to calcein-AM release CDC assays in which a similar protocol was used as in the calcein-AM release ADCC assay. MM cells were incubated with Ab in the presence of 10% pooled human complement serum.

Epitope mapping

For the Pepscan approach, epitope mapping was performed essentially as described by Teeling et al. (28). In short, overlapping 20-mer linear and 15-mer looped peptides were synthesized covering 138 aa at the C terminus of human CD38. From this panel of 42 Abs, daratumumab was selected, based on the sequence at the C terminus, single-looped peptides of different size were made covering region KNYRDPDKFLQCVKNPDESSCTSL, region CVHNLPQEKVQTLAEWVHGG, and region CLESLISSKRVQPSAKNYRC. Additionally, extra sets were designed to reconstitute double-looped regions that were comprised of SSRKNQFSCKNYR and EKVDQTLAEWVHGG. Native cytoines were replaced by alanines. Peptides were screened in an ELISA assay using credit card format mini-Pepscan cards.

For site-directed mutagenesis, mutations in the extracellular regions of human CD38 were introduced using the QuikChange XL site-directed mutagenesis kit, according to the manufacturer’s instructions (Strategene Europe, Amsterdam, The Netherlands). Mutagenesis was checked by sequencing (AGOWA, Berlin, Germany). Culture supernatants of transfected HEK-293F cells were used in ELISA. In this assay, anti-HA Ab (Sigma-Aldrich) was used as coating to capture HA-tagged CD38 proteins.
CD38 Abs were detected with HRP-conjugated goat anti-human IgG Abs (Jackson ImmunoResearch Laboratories, Newmarket, U.K.).

Mouse tumor xenograft models

Female, 8- to 10-wk-old C.B-17 SCID mice (C.B-17/IcrScid-Br), purchased from Charles River Laboratories (Maastricht, The Netherlands), or female 9- to 18-wk-old RAG2−/−/γc−/− mice (29) were housed in a barrier unit of the Central Laboratory Animal Facility (Utrecht, The Netherlands). Water and food were provided ad libitum. All experiments were approved by the Utrecht University animal ethics committee. Mice were checked three times a week for signs of discomfort and for general appearance.

For the Daudi tumor xenograft model, experiments were performed essentially as described by Bleeker et al. (19). Briefly, 2.5 × 106 Daudi-luc cells/mouse were injected i.c. Mice were treated with a single dose of CD38 mAb or isotype control Ab HuMab-KLH. Two different treatment settings (early and late treatment) were used. In the early treatment setting varying doses of Ab (0.01–100 μg/mouse i.p.; as described in the text) were administered within 1 h after tumor cell inoculation. In the late treatment setting Ab (10 μg/mouse i.p.) was administered 14 d after administration of cells. At weekly intervals mice were injected with synthetic γ-luciferin (acid form; Biothera, Haninge, Sweden) and luminescence was measured. For imaging, mice were anesthetized by i.p. injection of a mixture of ketamine, xylazine, and atropine before synthetic γ-luciferin was given i.p. (2.5 mg in 200 μl 10 mM Tris base/mouse). Mice were then placed in a light tight box and after 3 min imaging was started using a VersArray 1300B liquid nitrogen cooled charge-coupled device detector (Roper Scientific, Vianen, The Netherlands). Photons emitted from the luciferase system were counted over an exposure period of 5 min. Under illumination, black-and-white images were made for anatomical reference. MetaVue software (Universal Imaging/Molecular Devices, Downingtown, PA) was used for data collection and image analysis.

For the mouse UM9 tumor xenograft model, experiments were performed essentially as described by Rozemuller et al. (20). Briefly, 20 × 106 UM9-luc cells were injected i.v. RAG2−/−/γc−/− mice were treated with a single dose of 50 μg daratumumab or isotype control Ab 3 wk after tumor injection. Bioluminescent imaging using synthetic γ-luciferin was as described for the mouse Daudi tumor xenograft model.

Statistical analysis

Statistical analysis was performed by Student t test or by repeated measures one-way ANOVA followed by a Tukey post hoc test. In vivo differences were analyzed by two-way ANOVA followed by a Bonferroni post hoc test. Significance of the dose escalation experiment was tested with a normal linear mixed effect model. Treatments were considered significant when p < 0.05.

Results

Four competition groups

Human Ig transgenic mice were immunized with recombinant CD38 protein and CD38-transfected NIH 3T3 cells (NIH 3T3-CD38) until CD38-specific serum titer development. After fusion of spleen and lymph node cells with SP2/0 myeloma cells, hybridomas secreting CD38 Abs were cloned by limiting dilution. A panel of 42 human mAbs that specifically bound to CD38-transfected CHO cells (CHO-CD38) was identified. By competition binding experiments on CHO-CD38 cells, we were able to divide the mAbs into four competition groups (data not shown). Fig. 1A and 1B show binding of exemplary Abs from each competition group (designated competition groups 1–4) to CHO-CD38 cells and Daudi cells. Whereas good to excellent binding to CHO-CD38 and Daudi cells was found with Abs from competition groups 2, 3, and 4 (HM-003, daratumumab, and HM-029, respectively), somewhat weaker binding, particularly for Daudi cells, was found with the Ab from competition group 1 (HM-037). In a separate experiment, we demonstrated that daratumumab and HM-003 also bound to fresh MM tumor cells (Fig. 1C).

Induction of CDC

Tumor cell killing by CDC is considered an important mechanism of action for therapeutic Abs. The ability to induce CDC was tested in a first set of experiments with Daudi cells. After incubation of Daudi target cells with Abs and serum followed by staining with PI, killing was monitored by flow cytometry. Interestingly, only a single Ab in our panel of 42 human mAbs was able to induce CDC of Daudi cells. Fig. 1D shows that daratumumab from competition group 3 induced efficient complement-dependent lysis, with an EC50 of 0.16 μg/ml and achieving a maximal lysis of 56%, in contrast to prototypic Abs from the other competition groups. Fig. 1E shows that daratumumab was also unique among competition group 3 mAbs in its ability to induce CDC. Our data show that no complement-mediated cell lysis of Daudi cells was observed with any of the other mAbs from this group.

Next, we investigated whether daratumumab would also kill freshly isolated MM cells via CDC. To this end, mononuclear cells containing MM cells were obtained from bone marrow of 13 previously untreated or relapsed MM patients. After incubating these cells with normal human serum in the presence of Ab, complement-mediated lysis was determined. Daratumumab induced a concentration-dependent lysis of MM cells in 11 patients. The maximal lysis achieved for MM cells from each patient is shown in Table I (range, 10–90%; median, 51%). HM-003 was used as a control and did not induce CDC in any of the MM cell samples tested (data not shown).

Because the bone marrow microenvironment protects MM cells against cell death, we also studied whether daratumumab is able to effectively mediate CDC against MM cells in the presence of BMSCs. Importantly, daratumumab mediated effective CDC against XG-1 MM cells under these conditions (Fig. 1F), suggesting that daratumumab may also induce CDC in the bone marrow microenvironment.

Collectively, our results show that daratumumab is effective in killing MM cells via CDC.

Epitope mapping

The CDC experiments showed that the ability to induce complement-mediated killing is a unique characteristic of competition group 3 Ab daratumumab. To investigate whether this was linked to the epitope recognized, we first mapped daratumumab using a constrained peptide approach (Pepscan), which located its epitope to two β-strand–containing amino acids 233–246 and 267–280 of CD38 (Fig. 2A). We next investigated whether fine epitope heterogeneity existed between competition group 3 mAbs (data not shown). Mutant molecules incorporating all five amino acid differences between human and monkey CD38 located in the area shown in Fig. 2A were generated. Daratumumab, HM-025, HM-028, and HM-034 bound strongly to wild-type CD38 (Fig. 2B). Interestingly, replacing serine at position 274 for phenylalanine (S274F) completely abolished daratumumab binding, whereas binding of the other competition group 3 Abs was not affected (EC50 values: HM-025, 15 ng/ml; HM-028, 15 ng/ml; HM-034, 17 ng/ml) (Fig. 2C). Daratumumab thus seems to bind to a unique fine epitope on the CD38 molecule.

Induction of ADCC against MM cells

Ab-dependent killing via FcR-bearing effector cells is also considered an important mechanism of action for therapeutic Abs (30, 31). We assessed daratumumab-mediated ADCC against Daudi cells and a panel of drug-sensitive and drug-resistant MM cell lines in the presence of PBMCs enriched for NK cells. ADCC was measured by use of a calcine-AM release assay. Daratumumab induced a dose-dependent lysis of Daudi cells (EC50, ∼0.01 μg/
(n = 14) but not of CD38-negative U266 MM cells. Data in Table II show percentage lysis measured at a concentration of 0.1 μg/ml, a concentration at which all Abs had achieved maximal lysis (median, 39%; range, 6–65%). Specificity was confirmed in control experiments in which CD38-transfected CHO cells were efficiently killed by daratumumab-mediated ADCC (specific lysis, 30% at 0.1 μg/ml) but not parental, nontransfected, CHO cells (4% at 0.1 μg/ml) (data not shown).

Table I. Percentage maximum complement-mediated lysis induced by daratumumab in fresh MM cells obtained from tumor patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical Status</th>
<th>Lysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Relapse</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>Relapse</td>
<td>12*</td>
</tr>
<tr>
<td>3</td>
<td>Relapse</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>Untreated</td>
<td>81</td>
</tr>
<tr>
<td>5</td>
<td>Relapse</td>
<td>36*</td>
</tr>
<tr>
<td>6</td>
<td>Relapse</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>Relapse</td>
<td>68</td>
</tr>
<tr>
<td>8</td>
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<td>18</td>
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<tr>
<td>9</td>
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<td>53</td>
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<tr>
<td>10</td>
<td>Relapse</td>
<td>10*</td>
</tr>
<tr>
<td>11</td>
<td>Relapse</td>
<td>49</td>
</tr>
<tr>
<td>12</td>
<td>Relapse</td>
<td>84*</td>
</tr>
<tr>
<td>13</td>
<td>Relapse</td>
<td>39</td>
</tr>
</tbody>
</table>

Data show lysis at 1 μg/ml.

*Top value could not accurately be determined by GraphPad software (see Materials and Methods).

Next, we investigated the ability of daratumumab to induce killing of MM cells via ADCC obtained from four MM patients (patient status: one untreated, two relapsed, one refractory). The mononuclear cell fraction was enriched for tumor cells (range, 38–92%). Effector cells from eight different healthy donors were used (A–H). Data show mean lysis and SD. Lysis was determined by measurement of the percentage of PI-positive cells by flow cytometry. Designated epitope groups are given in brackets. E, CDC of Daudi cells after incubation with different Abs from competition group 3 in the presence of 10% pooled human serum from healthy donors as source of complement. Data show mean and SD after correction for lysis induced by anti-KLH. F, MM target cells were cultured in the absence (open bars) or presence (filled bars) of BMSCs in CDC assays. XG-1 cells were used as target cells. Anti-KLH (10 μg/ml) was used as negative control Ab. Data represent means of triplicates and SD.

In an additional experiment, daratumumab was added to freshly isolated tumor cells obtained from a patient with a CD38/CD138-positive, chemotherapy-refractory, plasma cell leukemia. Effector cells from three healthy donors were used. Daratumumab (EC50, 0.05 μg/ml; maximum lysis, 27%) and to a lesser extent HM-003 (EC50, 0.08 μg/ml; maximum lysis, 17%) induced dose-dependent lysis of these leukemic plasma cells by ADCC (Fig. 3C).
Daratumumab induces ADCC in CD38-expressing MM cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Daratumumab (% Lysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daudi</td>
<td>61.5</td>
</tr>
<tr>
<td>MM1R</td>
<td>43.2</td>
</tr>
<tr>
<td>MM1S</td>
<td>47.5</td>
</tr>
<tr>
<td>28BM</td>
<td>23.8</td>
</tr>
<tr>
<td>28PE</td>
<td>6.1</td>
</tr>
<tr>
<td>12BM</td>
<td>15.4</td>
</tr>
<tr>
<td>12PE</td>
<td>39.1</td>
</tr>
<tr>
<td>RPMI 8226</td>
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</tr>
<tr>
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<td>39.2</td>
</tr>
<tr>
<td>DOX40</td>
<td>39.3</td>
</tr>
<tr>
<td>L363</td>
<td>52.1</td>
</tr>
<tr>
<td>H929</td>
<td>65.2</td>
</tr>
<tr>
<td>INA6</td>
<td>18.0</td>
</tr>
<tr>
<td>LP-1</td>
<td>54.6</td>
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<td>27.2</td>
</tr>
<tr>
<td>U266</td>
<td>0</td>
</tr>
</tbody>
</table>

Data show maximal killing at 0.1 μg/ml obtained in a representative experiment. The experiment was repeated two to three times, depending on the cell line tested.
panel of Abs, the Ab daratumumab stood out. It was shown to bind to a unique region on the CD38 molecule, which facilitated killing of cell lines derived from various hematological tumors by ADCC, CDC, and apoptosis. Interestingly, daratumumab also potently induced killing of a large panel of patient MM tumor cells via ADCC and CDC. Our xenograft data suggest that this translates into a CD38 mAb with high anti-tumor activity in vivo.

Daratumumab was shown to induce CDC in a range of tumor cell samples from MM patients. Several studies have stressed the importance of CDC for therapeutic efficacy of Abs. For example, alemtuzumab (CD52 mAb) has been found to kill B cell tumor cells mainly via CDC (34), and the in vivo efficacy of rituximab (a chimeric CD20 Ab) is largely blocked by depletion of complement (35). In certain CLL patients, infusion of rituximab led to consumption of 90% of the patients’ hemolytic complement activities for periods of days to weeks (36). Moreover, the efficient killing of CLL cells by the human CD20 Ab ofatumumab is correlated with potent CDC (27, 28, 37). In contrast to rituximab, however, only

FIGURE 3. Daratumumab induces ADCC against MM cells. A, Percentage of maximum lysis of daratumumab-induced ADCC of tumor cells isolated from the bone marrow of four MM patient (patients are depicted by numbers). Cytotoxicity was determined by [51Cr] release. PBMCs were used as effector cells. For each patient sample, an experiment was performed with effector cells from two healthy donors (A–H). Filled bar, daratumumab; open bar, anti-KLH. B, Daratumumab induced dose-dependent ADCC against autologous patient MM cells (MM 1–9) as measured by calcein-AM release assays. CD38-purified myeloma cells from MM patients were used. C, A [51Cr]-release assay was performed using mononuclear cells from three healthy donors as effector cells. PBMCs of a patient with plasma cell leukemia were used as target cells. Data show mean ± SEM of three experiments. ■, Lysis induced by HM-003; ●, lysis induced by daratumumab; △, lysis induced by anti-KLH, the isotype control Ab. D, MM target cell line MM1R was cultured in the absence (open bars) or presence (filled bars) of BMSCs in calcein-AM release ADCC assays. NK cells from one healthy donor were used as effector cells in the ADCC assay. Anti-KLH (10 μg/ml) was used as negative control Ab.

FIGURE 4. Daratumumab inhibits outgrowth of CD38-expressing tumor cells in two different SCID xenograft models. A–C, SCID mice were inoculated with luciferase-expressing Daudi cells. A, Immediate treatment with daratumumab (100 μg/mouse). Control animals were euthanized on day 28 to avoid discomfort per protocol. Light emission from the back side of individual mice was collected at different time points. B, Immediate treatment (0.01–10 μg/mouse). Control mice were euthanized on day 35. Light emission collected from the back sides of mice was integrated over the total body areas of individual mice and plotted in time as measure of tumor mass development. A linear mixed effect model was used to analyze for significance. A significant difference was found between the control group (0 μg) versus all other groups, ***p < 0.001. C, Treatment was started on day 14 (10 μg/mouse). Mice were euthanized on day 35. Light emission collected from the back sides of mice was integrated over the total body areas of individual mice and plotted in time as measure of tumor mass development. D, RAG2−/−γc−/− mice were injected with luciferase-expressing UM-9 cells. Mice were treated with 50 μg daratumumab 3 wk after cell inoculation. Mice were euthanized on day 42. Data represent mean and SEM (six to eight mice per group). For data shown in C and D, a repeated-measures two-way ANOVA followed by a Bonferroni post hoc test on transformed data were used for statistical analysis. *p < 0.05; **p < 0.01; ***p < 0.001.
minimal amounts of complement were needed for ofatumumab to induce CDC (38). Data suggest that the positioning of ofatumumab after binding to its target may be ideal for complement activation and generation of the membrane attack complex (38). The same may hold true for daratumumab. In fact, induction of CDC was a very specific feature of daratumumab, as no other CD38 Ab from our panel was able to kill tumor cells in the presence of complement. Daratumumab was initially clustered in competition group 3 with a number of other CD38 Abs that all compete for binding. Interestingly, fine epitope mapping using constrained peptides and site-directed mutagenesis revealed that daratumumab binds to a unique fine epitope on CD38 distinct from the epitope recognized by the other competition group 3 Abs. Presumably, the binding of daratumumab to this unique CD38 epitope clusters and positions the Ab Fc in a way that facilitates more optimal binding and/or activation of complement proteins.

In MM patients, the complement system may be compromised due to decreased levels of components of the classical and alternative complement pathways (39–41). In an in vitro setting we could demonstrate that daratumumab is able to induce maximal complement-mediated lysis of MM cells in medium containing only 10% human serum. Furthermore, daratumumab-induced lysis could be restored in Clq-depleted serum by the addition of low amounts of Clq (representative of ~7% of normal Clq serum levels, data not shown). This suggests that daratumumab is still effective under complement-limiting conditions occurring in MM patients. Nevertheless, it will be important to monitor these aspects in the clinic.

In addition to CDC, daratumumab-induced ADCC of many different tumor cell lines with varying CD38 expression, patient MM tumor cells, and plasma cell leukemia cells. In line with this, it has been described that chimeric and humanized CD38 Abs were also able to induce ADCC in Burkitt lymphoma cell lines (15, 16). ADCC through IgG FcγR-bearing immune effector cells is thought to be an important mechanism of action by which therapeutic Abs exert their effect in vivo: in mice lacking expression of activating FcγR, cancer therapeutic Abs lose their effect on tumor growth (30), and in cancer patients FcγR polymorphisms directly impact therapeutic responses to Abs (31, 42, 43).

These data are in line with those of Stevenson et al. (15). They demonstrated that blood samples from myeloma patients who had undergone a variety of chemotherapeutic schedules were still able to mediate ADCC, resulting in lysis values that were not different from those obtained with effector cells from healthy controls (15). In fact, lytic activity of one of the major cell types involved in ADCC induction, NK cells, in peripheral blood of MM patients was comparable to normal control values and even increased in the bone marrow (44). Thus, engagement of immune effector cells in MM patients, and also in the bone marrow where tumor cells reside, is expected to occur. Our observations that daratumumab was capable of inducing ADCC against patient MM cells mediated by autologous PBMC effector cells (Fig. 3B) and that it effectively triggered myeloma cell lysis via ADCC as well as CDC in the presence of BMSCs are indeed significant. These results suggest that daratumumab should also be active in the patients’ bone marrow microenvironment, which usually supports MM growth, survival, and the development of drug resistance.

Recently, Benson et al. (45) showed that upregulated expression of the program death receptor-1 on NK cells of MM patients downmodulates the NK cell-mediated killing of MM cells via interaction with program death ligand-1. Although we show that daratumumab is able to induce ADCC of MM primary tumor cells in an autologous setting (Fig. 3B), we cannot exclude that interference with the program death receptor-1/program death ligand-1 axis, such as by downregulation of program death ligand-1 by lenalidomide treatment, could further enhance killing efficacy (23, 45–47).

The in vitro cytotoxic effects of daratumumab prompted us to investigate its activity in vivo. In an early treatment setting, daratumumab at a low dose of 0.5 μg/kg almost completely inhibited Daudi cell tumor growth. Our analysis estimates a half-maximal effective dose of 0.065 μg/kg body weight (95% C.I. 0.018–2.15 μg/kg). Importantly, in the late treatment settings (14 or 21 d after tumor establishment), a single dose of 0.5 or 2.5 mg/kg daratumumab suppressed tumor growth of Daudi cells or UM-9 cells, respectively, for at least 5 wk.

We observed unusually high efficacy of daratumumab in our Daudi tumor xenograft mouse model (half-maximal effective dose of 0.065 μg/kg). Although in this experiment treatment was performed at a low tumor load, this represents a dose at which the number of CD38 molecules expressed on the injected Daudi cells largely exceeds the number of administered daratumumab molecules. This indicates that daratumumab can already induce antitumor effects when only a small fraction of the CD38 molecules is occupied.

We should take into account that dosing requirements may be quite different in patients. Thus, pharmacokinetics can be affected by binding of daratumumab to CD38 molecules expressed on tumor cells and normal cells. Additionally, soluble CD38 molecules present in sera and ascites from myeloma patients (48) may affect Ab activity and clearance, although Stevenson et al. (15) did not observe binding of CD38 Abs to soluble CD38 in sera from multiple myeloma patients. The effect of high tumor load on pharmacokinetics has, for example, been demonstrated for CD20 Abs in patients with B cell tumors, where, after the first dose, plasma concentrations were much lower than expected. However, upon repeated dosing, CD20 Ab clearance decreases and effective serum Ab concentrations are obtained (49, 50). Therefore, we think that also for daratumumab the possible impact of cellular or soluble CD38 expression will be manageable in clinical applications.

In conclusion, we have generated a human IgG1 CD38 mAb, daratumumab, that binds the CD38 molecule at a unique epitope and is effective in killing tumor cells via multiple mechanisms of action, including CDC and ADCC. Importantly, daratumumab induced substantial autologous ADCC against patient MM cells and cell killing was still observed in the presence of BMSCs. Finally, daratumumab demonstrated a potent ability to disrupt MM and B cell tumor growth in two in vivo xenograft models. These data indicate that daratumumab is a therapeutic Ab with high potential for the treatment of CD38-positive MM tumors.

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