Preclinical Activity of JNJ-7957, a Novel BCMA×CD3 Bispecific Antibody for the Treatment of Multiple Myeloma, Is Potentiated by Daratumumab

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ABSTRACT

Purpose: Multiple myeloma (MM) patients with disease refractory to all available drugs have a poor outcome, indicating the need for new agents with novel mechanisms of action.

Experimental Design: We evaluated the anti-MM activity of the fully human BCMA×CD3 bispecific antibody JNJ-7957 in cell lines and bone marrow (BM) samples. The impact of several tumor- and host-related factors on sensitivity to JNJ-7957 therapy was also evaluated.

Results: We show that JNJ-7957 has potent activity against 4 MM cell lines, against tumor cells in 48 of 49 BM samples obtained from MM patients, and in 5 of 6 BM samples obtained from primary plasma cell leukemia patients. JNJ-7957 activity was significantly enhanced in patients with prior daratumumab treatment, which was partially due to enhanced killing capacity of daratumumab-exposed effector cells. BCMA expression did not affect activity of JNJ-7957. High T-cell frequencies and high effector:target ratios were associated with improved JNJ-7957–mediated lysis of MM cells. The PD-1/PD-L1 axis had a modest negative impact on JNJ-7957 activity against tumor cells from daratumumab-naïve MM patients. Soluble BCMA impaired the ability of JNJ-7957 to kill MM cells, although higher concentrations were able to overcome this negative effect.

Conclusions: JNJ-7957 effectively kills MM cells *ex vivo*, including those from heavily pretreated MM patients, whereby several components of the immunosuppressive BM microenvironment had only modest effects on its killing capacity. Our findings support the ongoing trial with JNJ-7957 as single agent and provide the preclinical rationale for evaluating JNJ-7957 in combination with daratumumab in MM.

Introduction

In the last decade, survival of multiple myeloma (MM) patients has markedly improved due to the introduction of immunomodulatory drugs (IMiD), proteasome inhibitors (PI), and the CD38-targeting antibody daratumumab. However, patients with disease refractory to these agents have a very poor outcome (1). Also, newly diagnosed MM patients with high-risk cytogenetic abnormalities have a reduced survival, when compared with patients with standard-risk disease (2). This indicates that there is still a need for new agents with novel mechanisms of action.

B-cell maturation antigen (BCMA) is a cell membrane–bound tumor necrosis factor receptor family member and expressed on a small subset of normal mature B cells, as well as normal plasma cells and MM cells. BCMA is involved in differentiation of normal B cells to plasma cells (3, 4). In addition, binding of a proliferation-inducing ligand (APRIL) or B-cell activating factor (BAFF), which has 1,000-fold lower affinity than APRIL, to BCMA promotes MM growth and induces immunosuppression in the bone marrow (BM) microenvironment (5–7). The selective expression of BCMA on normal and malignant plasmablasts and plasma cells renders it an attractive target for novel MM treatment strategies (8, 9). Indeed, BCMA antibody–drug conjugates and BCMA chimeric antigen receptor (CAR) T cells show much promise in patients with advanced MM (10–14). Furthermore, preclinical studies have demonstrated that BCMA×CD3 bispecific antibodies and bispecific T-cell engagers (BiTE) have marked anti-MM activity (9, 15, 16). In addition, preliminary results from a phase I study show encouraging single-agent activity of AMG420, a BCMA-targeting BiTE (17). These bispecific antibodies and BiTEs redirect T cells to the BCMA-positive tumor cell, which is followed by perforin and granzyme release, and ultimately MM cell death. Killing of tumor cells by redirected T cells is independent of T-cell receptor specificity. However, MM patients with advanced disease have a more immunosuppressive BM microenvironment, including increased regulatory T-cell (Treg) counts, as well as upregulation of inhibitory immune checkpoints such as programmed death ligand-1 (PD-L1) on MM cells and programmed cell death-1 (PD-1) on T cells, which may limit the activity of bispecific antibodies in these patients (18, 19). In addition, prior immunosuppressive anti-MM therapies may hamper T-cell function and affect the activity of these T-cell–redirecting therapies (20).

Interestingly, next to its classic Fc-dependent immune effector functions, daratumumab also has immunomodulatory effects through

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Translational Relevance

There is a need for new agents with novel mechanisms of action in multiple myeloma (MM). B-cell maturation antigen (BCMA) has been identified as a novel target for MM treatment, due to its restricted expression on plasma cells. We show that the novel BCMA×CD3 bispecific antibody, JNJ-7957, induces potent T-cell–mediated killing of MM cell lines. JNJ-7957 also effectively eliminated primary MM cells in 48 of 49 bone marrow (BM) samples obtained from both newly diagnosed and heavily pretreated MM patients. Interestingly, JNJ-7957 activity was significantly enhanced in patients with prior daratumumab treatment, which was partially due to enhanced killing capacity of daratumumab-exposed effector cells. The immunosuppressive BM microenvironment had only limited inhibitory effects on JNJ-7957 activity. Altogether, these data strengthen the preclinical rationale for the ongoing clinical trial evaluating JNJ-7957. In addition, our data indicate that clinical evaluation of the combination of daratumumab and JNJ-7957 is warranted.

elimination of immune suppressor cells, induction of T-cell expansion, and enhancement of the cytolytic capacity of T cells (21–26), which may potentially improve the efficacy of T-cell–redirecting agents to kill MM cells.

We therefore evaluated in this study the preclinical activity of the novel fully human BCMA×CD3 bispecific antibody (JNJ-7957), which was developed using the Genmab DuoBody technology and has silenced Fc function to eliminate Fc-dependent immune effector functions (27). The antitumor efficacy was tested in MM cell lines and in BM samples obtained from patients with newly diagnosed (ND) or relapsed/refractory (RR) MM, including heavily pretreated patients with disease refractory to IMiDs, PIs, and CD38 antibodies. We analyzed the impact of several host and tumor characteristics on *ex vivo* JNJ-7957 response. Furthermore, we evaluated the potential ability of daratumumab to improve the efficacy of JNJ-7957 to kill MM cells by virtue of its immunomodulatory effects.

Materials and Methods

Patients

Ex vivo efficacy of JNJ-7957 was assessed in 55 BM aspirates obtained from patients with NDMM ($n = 11$), daratumumab-naïve RRMM ($n = 21$), daratumumab-refractory RRMM ($n = 17$), and primary plasma cell leukemia (pPCL; $n = 6$). In these samples, we concurrently evaluated several tumor characteristics, including BCMA expression levels, as well as immune cell composition. The samples were analyzed within 24 hours after BM aspiration.

To test the impact of daratumumab treatment on JNJ-7957, sequential BM samples were obtained from 8 RRMM patients treated in the daratumumab/ATRA study (NCT02751255), directly before initiation of daratumumab monotherapy and immediately at the time of daratumumab-refractory disease. In the same study, we obtained sequential peripheral blood (PB) samples from 13 patients, directly before initiation of daratumumab monotherapy and during treatment with daratumumab monotherapy. Briefly, in the daratumumab/ATRA study, patients had MM requiring systemic treatment and were relapsed from or refractory to ≥2 prior lines of therapy. During the first part of this study, daratumumab monotherapy was given according to the approved dose and schedule (16 mg/kg weekly for 8 weeks, then every 2 weeks for 16 weeks, and every 4 weeks until progression).

The study site ethics committee approved the protocols, which were conducted according to the principles of the Declaration of Helsinki, the International Conference on Harmonization, and the Guidelines for Good Clinical Practice. All patients gave written informed consent.

Antibodies and reagents

JNJ-7957 (JNJ-64007957) and daratumumab were provided by Janssen Pharmaceuticals. CNTO7008 (CD3 × null), BC3B4 (BCMA × null), and CNTO3930 (IgG isotype control) were used as control antibodies and provided by Janssen Pharmaceuticals. Recombinant human BCMA and recombinant human APRIL were obtained from Sigma-Aldrich. Blinatumomab (Amgen) was obtained via the clinical pharmacy of Amsterdam University Medical Center.

Flow-cytometric analysis of BM and blood samples from MM patients

BM-localized MM cells were identified and analyzed for cell-surface marker expression levels by staining 1.0×10^6 cells/mL with CD138 PE, CD56 PC7, CD45 Krome Orange (all Beckman Coulter), CD269 (BCMA) APC (BioLegend), CD274 (PD-L1) BV421, and CD19 APC-H7 (both Becton Dickinson). To assess CD38 expression irrespective of ongoing or recent daratumumab treatment, cells were also stained with HuMax-003 FITC, which binds to a CD38 epitope distinct from the epitope bound by daratumumab (Genmab/Janssen Pharmaceuticals).

Immune cell subsets in whole PB or BM aspirates were identified and analyzed for cell-surface marker expression levels by staining 1.0×10^6 cells/mL with CD45 Krome Orange, CD56 PC7 (both Beckman Coulter), CD14 APC-H7, CD19 APC-H7, CD3 V450, CD4 APC-H7 or PE, CD8 FITC, CD45-RA APC, CD127 PE.Cy7, CD62L PE, CD274 (PD-1) BV421, CD16 APC, HLA-DR APC-H7 (all Becton Dickinson), CD25 PE (Dako), and CD38 HuMax-003 FITC. Naïve T cells were defined as $CD45RA^+CD62L^+$, effector memory (EM) T cells as $CD45RA^-$ $CD62L^-$, central memory (CM) T cells as $CD45RA^-CD62L^+$, and terminally differentiated EM T cells expressing CD45RA (TEMRA) as $CD45RA^+CD62L^-$ (28).

Using a separate antibody panel, $CD38^+$ Tregs ($CD3^+CD4^+$ $CD127^{dim}CD25^+CD38^+$) and Bregs ($CD19^+CD24^+CD38^+$) were identified in sequential BM samples obtained from 8 daratumumab-treated patients, before start of daratumumab and at the time of daratumumab-refractory disease, as described previously (21).

Flow cytometry was performed using a 7-laser LSRFORTESSA (Becton Dickinson). Fluorescent-labeled beads (CS&T beads, Becton Dickinson) were used daily to monitor the performance of the flow cytometer and verify optical path and stream flow. This procedure enables controlled standardized results and allows the determination of long-term drifts and incidental changes within the flow cytometer. No changes were observed which could affect the results. Compensation beads were used to determine spectral overlap, and compensation was automatically calculated using Diva software. Flow cytometry data were analyzed using FACSDiva software.

Flow cytometry–based *ex vivo* lysis assays in BM-MNCs

BM-MNCs derived from MM patients, containing tumor cells as well as autologous effector cells, were used in flow cytometry–based lysis assays. Sample viability at start of the assays, assessed using

7-AAD (Becton Dickinson), was more than 95%. The percentage of viable MM cells was 14.9% (range, 1.0%–74.8%) of BM-MNCs at baseline, and 10.2% (range, 1.0–66.0%) after 48 hours in solvent control-treated conditions. BM-MNCs were incubated in RPMI + 10% fetal bovine serum with control antibodies (4.0 μg/mL) or JNJ-7957 (0.0064–4.0 μg/mL; corresponding to 0.043-26.68 nmol/L; 1 μg/mL = 6.67 nmol/L) and/or daratumumab (10 μg/mL) in 96-well U-bottom plates for 48 hours. The survival of primary CD138$^+$ MM cells in the BM-MNCs was determined by flow cytometry as previously described (29–32). Briefly, surviving MM cells were enumerated by single platform flow-cytometric analysis of CD138$^+$ cells in the presence of Flow-Count Fluorospheres (Beckman Coulter) and LIVE/DEAD Fixable Dead Cell Stain Near-IR fluorescent reactive dye (Invitrogen). The percentage of lysis induced

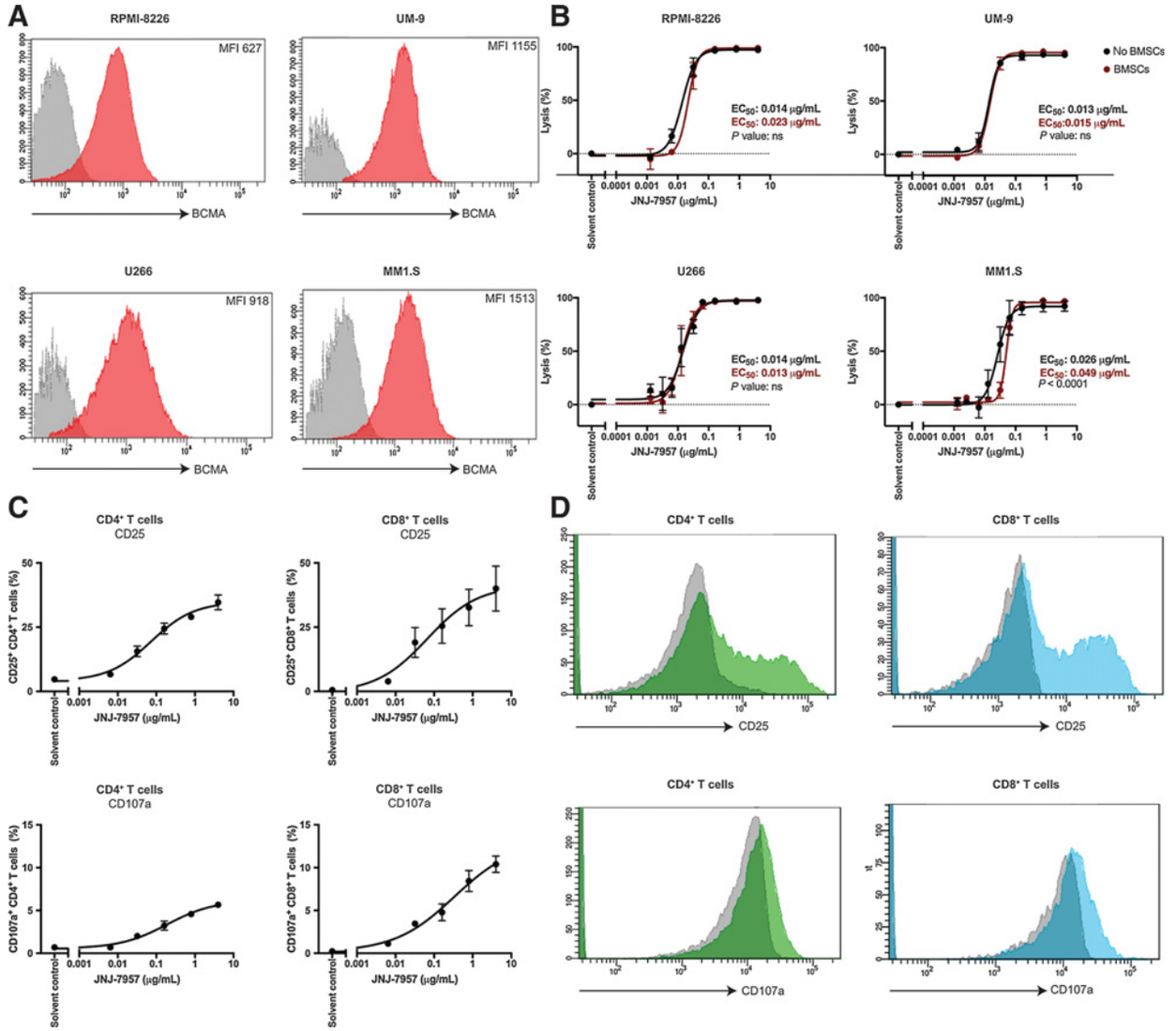

Figure 1.

JNJ-7957–mediated lysis of MM cell lines. **A,** Representative flow cytometry histogram overlays depicting BCMA cell surface expression on four MM cell lines (RPMI-8226, UM9, U266, and MM1.S; red histogram), compared with isotype control (gray histogram). BCMA MFI is provided. **B,** These four MM cell lines were incubated with solvent control or JNJ-7957 (0.00128–4.0 μg/mL) and PB-MNCs obtained from healthy donors as effector cells at a ratio of 10:1 for 48 hours, in the presence or absence of BMSCs. MM cell lysis was assessed using a BLI-based cytotoxicity assay. Data represent mean MM cell lysis ± SEM, of 3 or 4 independent experiments performed in duplicate. EC_{50} values are provided for both conditions. Differences in JNJ-7957–mediated tumor cell lysis in the presence or absence of BMSCs were calculated using nonlinear regression analysis. **C,** RPMI-8226 cells were incubated with PB-MNCs from healthy donors and increasing concentrations of JNJ-7957 (0.0064–4.0 μg/mL) for 48 hours, after which cell surface expression of activation marker CD25 and degranulation marker CD107a on CD4$^+$ and CD8$^+$ T cells were determined by flow cytometric analysis. Data represent mean ± SEM of 2 independent experiments performed in duplicate. **D,** Representative flow cytometry histogram overlays depicting cell surface expression of activation marker CD25 and degranulation marker CD107a on CD4$^+$ (green histogram) and CD8$^+$ (blue) T cells treated with 4.0 μg/mL JNJ-7957 for 48 hours, compared with solvent control-treated cells (gray). MFI, median fluorescence intensity; ns, not significant; SEM, standard error of mean.

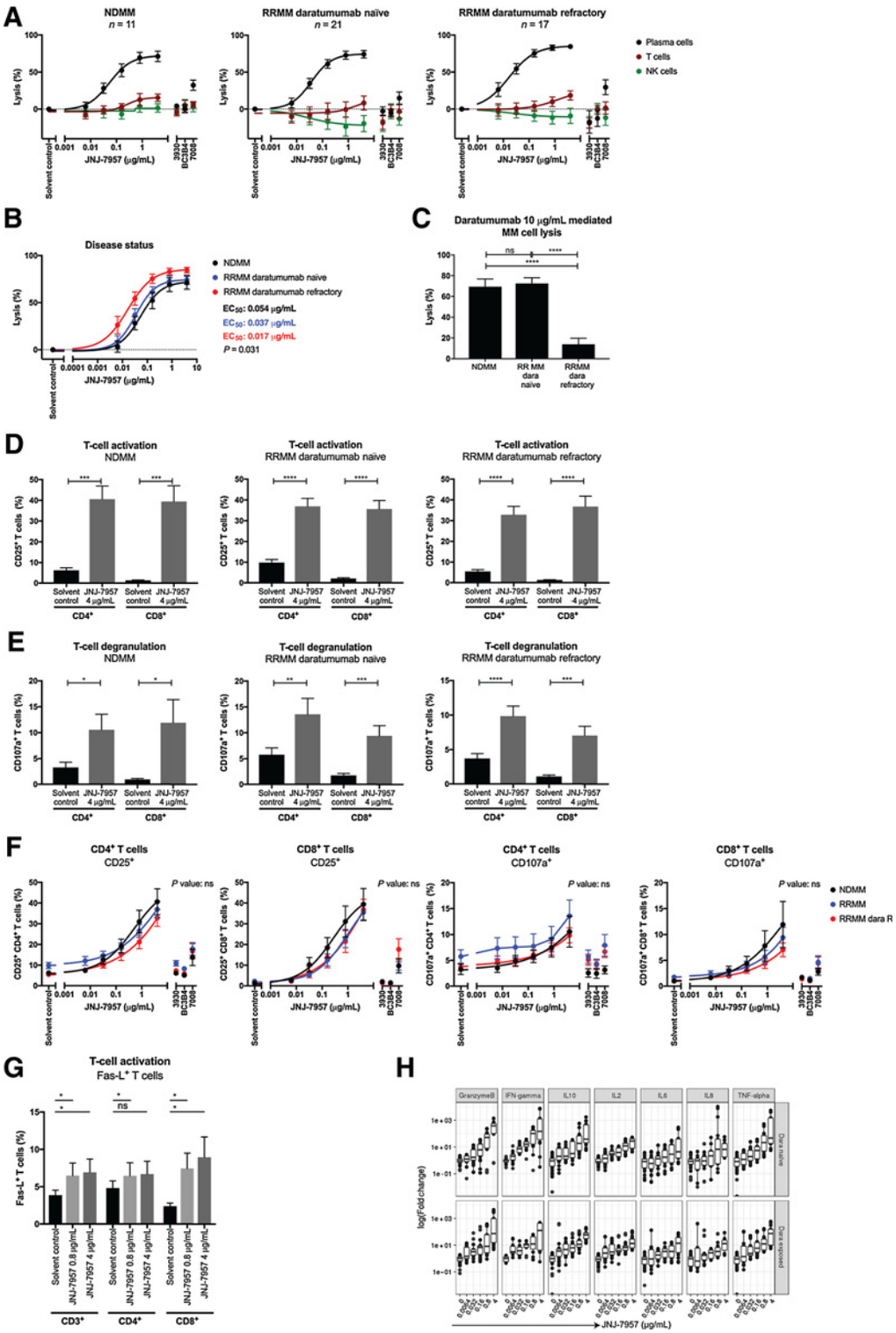

Figure 2.

JNJ-7957–mediated lysis of primary MM cells. **A,** BM-MNCs obtained from 11 NDMM, 21 daratumumab-naïve RRMM, and 17 daratumumab-refractory RRMM patients were incubated with JNJ-7957 (0.0064–4.0 μg/mL) or control antibodies 3930, BC3B4, and 7008 (4.0 μg/mL) for 48 hours, after which surviving $CD138^+$ tumor cells, as well as T and NK cells, were enumerated using flow-cytometric analysis. Lysis (y-axis) of plasma cells (black), T cells (dark red), and NK cells (green) is presented in the graphs. Negative lysis values indicate that cell numbers are higher, when compared with control. (*Continued on the following page.*)

by JNJ-7957 and/or daratumumab was calculated using the following formula: % lysis MM cells = 1 − (absolute number of surviving $CD138^+$ cells in treated wells/absolute number of surviving $CD138^+$ cells in untreated wells) × 100%, as described previously (31).

The JNJ-7957–induced activation and degranulation of $CD4^+$ and $CD8^+$ T cells were analyzed by the flow-cytometric detection of CD25 PE (DAKO) and CD107a BV421 (Becton Dickinson) cell-surface expression, respectively. In a subset of patients, $CD4^+$ and $CD8^+$ T cells were also evaluated for JNJ-7957–induced CD178 expression [Fas Ligand (FasL); Becton Dickinson] by flow cytometry.

Statistical analysis

Comparisons between continuous variables were performed using two-tailed (paired) Student *t* test, or Mann–Whitney U test, or Wilcoxon matched-pairs signed-rank test in case the data did not follow a normal distribution. The Pearson X^2 test was used for categorical data. Data were visualized using mean and standard error of mean (SEM) to compare differences between means and to facilitate visual comparison of means. Linear mixed models were performed to study the dose–response relationship. Two-level models were used with JNJ-7957 concentration nested within patients to account for the dependency of the repeated dose measurements. To account for the nonlinear relationship between dose and response, JNJ-7957 concentration, together with a squared version of the concentration, was included in the model. Univariate analyses were performed to study the relationship between tumor or immune characteristics and response to JNJ-7957 treatment. Apart from cytogenetic risk status, all parameters were analyzed as continuous variables, whereas in graphs patients were dichotomized based on the median values of each variable to visualize the impact of each variable on JNJ-7957 response. Only factors that had a significance of $P < 0.10$ in the univariate analyses were included in the multivariate analysis to assess independent determinants of response.

In case of combinatorial treatment of JNJ-7957 and daratumumab, expected lysis values were calculated, using the following formula that assumes that there is an additive effect between the combined agents: % expected lysis = (% lysis with JNJ-7957 + % lysis with daratumumab) – (% lysis with JNJ-7957 × % lysis with daratumumab), as described before (30, 33, 34). Paired Student *t* tests were used to test the statistical difference between the observed and expected values. The hypothesis of additivity was rejected, and synergy was concluded, if the observed values were significantly higher than the expected values. Half maximal effective concentration (EC_{50}) values were compared using one-way ANOVA. Statistical analyses were performed in GraphPad Prism (version 7) and R (version 3.5). *P* values below 0.05 were considered significant.

Details and additional methods are presented in the Supplementary Data.

Results

BCMA×CD3 effectively kills MM cell lines in both the absence and presence of stromal cells

To evaluate the anti-MM activity of JNJ-7957, we first performed BLI-based cytotoxicity assays by incubating 4 luciferase (LUC)-transduced MM cell lines (RPMI-8226, UM9, U266, and MM1.S), which have different expression levels of BCMA (**Fig. 1A**), with serial concentrations of JNJ-7957 for 48 hours in the presence of healthy donor–derived PB mononuclear cells (MNC) at a ratio of 10:1. There was dose-dependent lysis of MM cells and near-complete elimination of the MM cells with 0.16 μg/mL JNJ-7957, irrespective of the BCMA expression of the cell lines (**Fig. 1B**). Consistent with a T-cell–dependent mode of action, treatment with the bispecific antibody resulted in activation and degranulation of both $CD4^+$ and $CD8^+$ T cells in a dose-dependent manner, as evidenced by the increased cell-surface expression of CD25 and CD107a, respectively (**Fig. 1C** and **D**). JNJ-7957 also effectively killed MM cells at lower PB-MNC to MM cell ratios after both 48- and 96-hour incubations (Supplementary Fig. S1).

Bone marrow stromal cells (BMSC) protect MM cells against various anti-MM agents including daratumumab (35) and MM-reactive T cells (36). We therefore explored the potential impact of BMSC-MM cell interactions on the efficacy of JNJ-7957. As previously demonstrated (36), BMSCs significantly inhibited the lysis of UM9 cells by an MM-reactive cytotoxic T-cell clone (Supplementary Fig. S2). However, as depicted in **Fig. 1B**, the activity of JNJ-7957 against the MM cell lines RPMI-8226, UM9, and U266 was not affected by the presence of BMSCs. Although JNJ-7957–mediated MM cell lysis was modestly inhibited by BMSCs in MM1.S cells at lower concentrations ($P < 0.0001$), this effect was completely abrogated by increasing the JNJ-7957 dose.

(*Continued.*) Data represent mean ± SEM; all experiments were performed in duplicate. **B,** JNJ-7957–mediated tumor cell lysis in samples obtained from NDMM (black), daratumumab-naïve (blue), and daratumumab-refractory RRMM patients (bright red) was compared using nonlinear regression analysis. Data represent mean ± SEM. EC_{50} values are provided for each category of MM patients. **C,** BM-MNCs from NDMM ($n = 11$), daratumumab-naïve RRMM ($n = 17$), and daratumumab-refractory RRMM ($n = 21$) were incubated with daratumumab 10 μg/mL for 48 hours, after which surviving $CD138^+$ tumor cells were enumerated using flow-cytometric analysis. Data represent mean ± SEM. *P* values between indicated groups were calculated using unpaired Student *t* test. **D,** Bar graphs depict activation of $CD4^+$ and $CD8^+$ T cells treated with solvent control or 4.0 μg/mL JNJ-7957 in NDMM, daratumumab-naïve, and daratumumab-refractory RRMM patient samples. Data represent mean ± SEM. *P* values were calculated using paired Student *t* test. **E,** Bar graphs depict degranulation of $CD4^+$ and $CD8^+$ T cells treated with solvent control or 4.0 μg/mL JNJ-7957 in NDMM, daratumumab-naïve, and daratumumab-refractory RRMM patient samples. Data represent mean ± SEM. *P* values were calculated using paired Student *t* test. **F,** JNJ-7957–mediated activation and degranulation of $CD4^+$ and $CD8^+$ T cells were assessed after a 48-hour culture by flow-cytometric analysis of CD25 and CD107a cell-surface expression, respectively. Data represent mean ± SEM; experiments were performed in duplicate. T-cell activation and degranulation were compared between the three patient categories [NDMM (black), daratumumab-naïve RRMM (blue), and daratumumab-refractory RRMM (bright red)] using nonlinear regression analysis. **G,** Bar graphs depict the frequency of $FasL^+$ total ($CD3^+$), $CD4^+$, and $CD8^+$ T cells treated with solvent control or JNJ-7957 (0.8 or 4.0 μg/mL) in BM samples obtained from 4 MM patients; experiments were performed in duplicate. Data represent mean ± SEM. Differences between indicated groups were calculated using paired Student *t* test. **H,** Granzyme B, IFNγ, IL10, IL2, IL6, IL8, and tumor necrosis factor-α were measured in the cell culture supernatants of BM-MNCs treated with JNJ-7957 (0.0064–4.0 μg/mL) for 48 hours. BM-MNCs were obtained from daratumumab-naïve ($n = 7$) and daratumumab-refractory ($n = 7$) patients. Dots represent individual data; boxes represent first and third quartiles; the line represents the median. Data were normalized to solvent control-treated cells. 3930, isotype control antibody; 7008, CD3×null control antibody; BC3B4, BCMA×null control antibody; dara, daratumumab; EC_{50}, half maximal effective concentration; ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

BCMA×CD3 effectively kills primary MM cells obtained from NDMM and heavily pretreated RRMM patients

MM cells become increasingly resistant toward anti-MM agents during the patient's treatment course, and different therapies may also affect T-cell numbers and function. To evaluate whether clinical resistance to bortezomib, lenalidomide, and/or daratumumab has an impact on the activity of JNJ-7957, we analyzed JNJ-7957–mediated killing of BM-localized MM cells from NDMM and RRMM patients in flow cytometry–based cytotoxicity assays. The characteristics of these patients are shown in Supplementary Table S1. In these experiments, BM-MNCs, containing tumor cells as well as autologous immune cells, were treated with JNJ-7957 for 48 hours.

JNJ-7957 effectively induced MM cell lysis in 48 out of 49 samples obtained from MM patients. MM cell lysis was observed in both untreated and heavily pretreated patients: in 10 of 11 NDMM samples, in all daratumumab-naïve RRMM patients ($n = 21$; median of 3 prior therapies; 86% lenalidomide refractory and 48% bortezomib refractory; last line of therapy contained an IMiD in 71% of the patients, PI in 10%, and IMiD plus PI in 19%), and in all daratumumab-refractory patients ($n = 17$; median of 6 prior therapies; 100% lenalidomide refractory and 94% bortezomib refractory; **Fig. 2A**). Interestingly, JNJ-7957–mediated tumor cell lysis was significantly better in the daratumumab-refractory BM samples, when compared with daratumumab-naïve patient samples ($P = 0.031$; **Fig. 2B**). As expected, there was a marked reduction in *ex vivo* anti-MM activity of daratumumab in BM samples obtained from daratumumab-refractory patients, when compared with activity of daratumumab against daratumumab-naïve MM cells ($P < 0.0001$; **Fig. 2C**).

The CD3 × null and BCMA × null control antibodies showed significantly lower activity in the different patient samples, when compared with JNJ-7957, indicating the requirement for cross-linking of the MM cell and the effector T cells, as well as absence of a direct effect of BCMA blockade. In addition, BCMA-negative $CD4^+$ T cells and $CD8^+$ T cells were not killed by JNJ-7957 in the three different patient populations (**Fig. 2A** and Supplementary Fig. S3). Furthermore, natural killer (NK)–cell survival was not affected in samples from NDMM patients, whereas there was a small increase in NK cell counts in samples from RRMM patients (**Fig. 2A**).

BCMA×CD3 induces activation and degranulation of $CD4^+$ and $CD8^+$ T cells, as well as production of proinflammatory cytokines in BM samples from MM patients

To assess the involvement of T cells in the effect of JNJ-7957–mediated lysis of MM cells in the BM samples, we assessed T-cell activation and degranulation, as well as release of cytokines and granzyme B after 48 hours. Similar to the experiments with cell lines, JNJ-7957–mediated lysis of primary MM cells was associated with a significant increase in the percentage of activated $CD4^+$ and $CD8^+$ T cells, as evidenced by enhanced expression of CD25 (**Fig. 2D**). JNJ-7957 treatment was also associated with significant degranulation of $CD4^+$ and $CD8^+$ T cells, as determined by cell-surface CD107a expression (**Fig. 2E**). There was no difference in the extent of T-cell activation and degranulation between NDMM, daratumumab-naïve RRMM, and daratumumab-refractory MM patients (**Fig. 2F**). Furthermore, JNJ-7957 treatment significantly increased the frequency of $FasL^+$ T cells, suggesting that Fas-FasL–based interactions may also contribute to MM cell lysis (**Fig. 2G**). As expected, there was no activation or degranulation of NK cells in these assays (Supplementary Fig. S4).

We also assessed levels of granzyme B and various cytokines in the supernatant of the JNJ-7957–treated BM-MNCs from daratumumab-naïve and daratumumab-refractory RRMM patients. JNJ-7957–mediated T-cell activation resulted in a dose-dependent increase in levels of granzyme B, IFNγ, IL2, IL6, IL8, IL10, and TNFα (**Fig. 2H**).

The efficacy of BCMA×CD3 is not associated with BCMA expression or cytogenetic risk status, but shows an inverse correlation with PD-L1 expression in daratumumab-naïve RRMM samples

To further evaluate the heterogeneity in response to JNJ-7957 in patient samples, we analyzed the impact of baseline tumor characteristics on JNJ-7957 activity. Across all BM samples, the efficacy of JNJ-7957 was not associated with cell-surface expression levels of BCMA and PD-L1, or with cytogenetic risk status (**Fig. 3A**). However, when we analyzed the three patient categories separately, we observed that BCMA and PD-L1 expression levels were significantly higher in RRMM patients, compared with NDMM patients, irrespective of daratumumab exposure (Supplementary Fig. S5A). Although patient numbers are small, the activity of JNJ-7957 was inversely correlated with PD-L1 expression levels in daratumumab-naïve RRMM patients ($P = 0.045$; Supplementary Fig. S5Bii). Other tumor cell characteristics were not associated with JNJ-7957 activity in these subgroup analyses (Supplementary Fig. S5B).

The efficacy of BCMA×CD3 is associated with T-cell frequency and effector-to-target cell (E:T) ratio

Next, we analyzed whether part of the heterogeneity in response to JNJ-7957 could be explained by naturally occurring differences in the composition of the immune cells in these BM aspirates. In the whole group of patients, a high T-cell frequency ($P = 0.034$) and high E:T ratio ($P = 0.029$) were associated with superior JNJ-7957–mediated lysis of MM cells (**Fig. 3B**). Similar observations were made when $CD4^+$ and $CD8^+$ T cells were analyzed separately (data not shown). Other immune parameters did not affect JNJ-7957–mediated MM cell lysis (**Fig. 3B**).

In subgroup analyses, we observed in RRMM patients a significantly higher frequency of Tregs and activated T cells (defined by expression of HLA-DR), and a lower frequency of naïve T cells, when compared with NDMM patients. In addition, daratumumab-refractory patient samples contained significantly more TEMRA T cells than daratumumab-naïve samples (Supplementary Fig. S6A). However, frequencies of activated, naïve, CM, EM, or TEMRA T cells were not associated with response to JNJ-7957 in this subgroup analysis (Supplementary Fig. S6B). A high frequency of Tregs impaired MM cell lysis by low-dose JNJ-7957 (0.0064 and 0.032 μg/mL) in samples from RRMM patients, which was overcome by higher JNJ-7957 concentrations (overall not statistically significant). The proportion of $PD\text{-}1^+$ T cells and E:T ratio was similar in the three patient groups. Only in NDMM patients, a low frequency of T cells ($P = 0.010$) and a high frequency of $PD\text{-}1^+$ T cells ($P = 0.048$) impaired JNJ-7957–mediated lysis of MM cells (Supplementary Fig. S6B).

In order to determine independent predictive factors for response to JNJ-7957, we performed a multivariate analysis. Treatment, tumor, and immune system characteristics (prior daratumumab exposure, T-cell frequency, and E:T ratio) that showed a significance of $P < 0.10$ in the univariate analyses were included in this analysis. Prior daratumumab exposure ($P = 0.034$) and T-cell frequency ($P = 0.024$) were independent determinants of response to JNJ-7957.

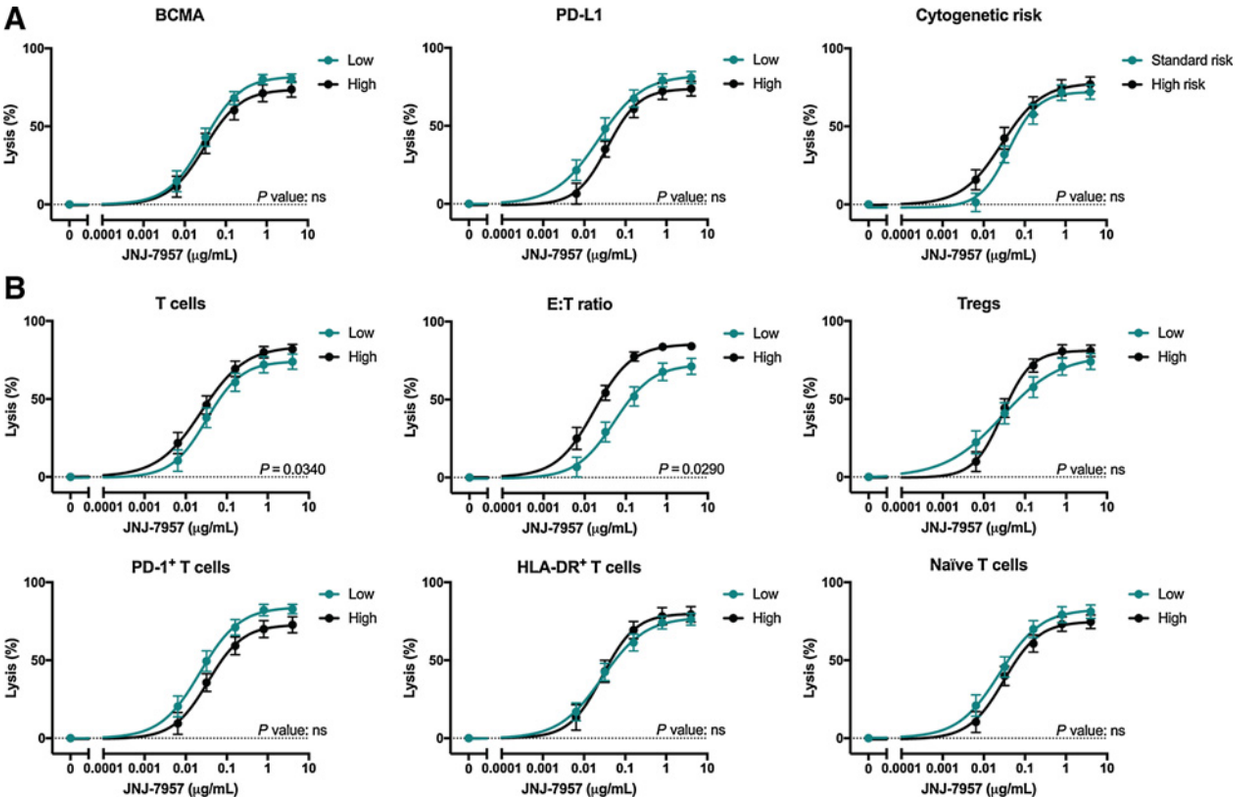

Figure 3.

Effect of tumor characteristics and T-cell composition on JNJ-7957–mediated MM cell lysis. **A,** Dose–response curves for JNJ-7957–mediated MM cell lysis according to median BCMA (MFI 179) and PD-L1 (MFI 981) expression levels, as well as presence of high-risk cytogenetic abnormalities. Data represent mean ± SEM. Differences in JNJ-7957–mediated MM cell lysis according to BCMA and PD-L1 expression (assessed as continuous variables) or presence of high-risk cytogenetic abnormalities were calculated using nonlinear regression analyses. **B,** Dose–response curves of JNJ-7957–mediated MM cell lysis according to the median percentage of T cells (14.8%), Tregs (11.32%), PD-1$^+$ T cells (23.51%), HLA-DR$^+$ T cells (27.14%), and naïve T cells (11.19%), as well as E:T ratio (3.18) at baseline. Data represent mean ± SEM. Differences in JNJ-7957–mediated MM cell lysis were calculated using nonlinear regression analyses with all immune characteristics analyzed as continuous variables. Naïve T cells were defined as CD45RA$^+$CD62L$^+$, effector memory (EM) T cells as CD45RA$^-$ CD62L$^-$, central memory (CM) T cells as CD45RA$^-$CD62L$^+$, and terminally differentiated effector memory T cells expressing CD45RA (TEMRA) as CD45RA$^+$CD62L$^-$. dara, daratumumab; HLA-DR, human leukocyte antigen—DR isotype; MFI, median fluorescence intensity; ns, not significant.

Pretreatment with daratumumab improves the efficacy of JNJ-7957 in serial BM samples

Because daratumumab exposure was independently associated with improved JNJ-7957–mediated MM cell lysis, we further evaluated prior daratumumab treatment as determinant of response to JNJ-7957. To this end, we first tested JNJ-7957 efficacy in serial BM aspirates from 8 RRMM patients, taken at the time of initiation of daratumumab monotherapy and at the time of refractory disease. In these serial samples, there was a significant improvement in JNJ-7957–induced killing of MM cells from aspirates taken at the time of daratumumab resistance, compared with pretreatment samples ($P = 0.0004$; median duration of daratumumab treatment 3 months; range, 1–7 months; **Fig. 4A**). Following JNJ-7957 treatment, there was no significant difference in the percentage of activated or degranulated T cells between samples from daratumumab-naïve or -refractory patients (Supplementary Fig. S7A). Similar to our prior findings (21), comparison of these paired BM samples prior to JNJ-7957 treatment revealed that *in vivo* daratumumab treatment significantly reduced the frequency of CD38$^+$ Tregs and Bregs, and increased the frequency of CD8$^+$ T cells (**Fig. 4B**). There were no significant differences in E:T ratio; frequency of total Tregs, PD-1$^+$ T cells or HLA-DR$^+$ T cells; and BCMA or PD-L1 expression on the tumor cells (data not shown).

In vivo daratumumab pretreatment of effector cells augments the *ex vivo* efficacy of JNJ-7957

We hypothesized that the improved JNJ-7957–mediated MM cell killing after *in vivo* daratumumab treatment is the result of these immunomodulatory effects. To test this, we obtained serial blood samples from 13 RRMM patients at the time of initiation of daratumumab monotherapy and during daratumumab treatment (median duration of daratumumab treatment 7 months; range, 2–19 months). RPMI-8226 cells were treated with JNJ-7957 in the presence of PB-MNCs from these patients. JNJ-7957–mediated MM cell lysis was superior in the presence of the daratumumab-exposed PB-MNCs, when compared with the daratumumab-naïve PB-MNCs ($P < 0.0001$; **Fig. 4C**). This could not be explained by differences in activation or degranulation between daratumumab-naïve and daratumumab-exposed T cells following treatment with JNJ-7957 (Supplementary Fig. S7B).

Daratumumab treatment enhances *ex vivo* efficacy of blinatumomab

To assess if daratumumab treatment is also beneficial for other T-cell–redirecting therapies, we treated CD19$^+$ Raji cells with blinatumomab, an FDA-approved CD19×CD3 BiTE for the treatment of acute lymphoblastic leukemia (37), using paired daratumumab-naïve and exposed PB-MNCs from 11 of these 13 MM patients. Similar to our observations with JNJ-7957, the activity of blinatumomab was

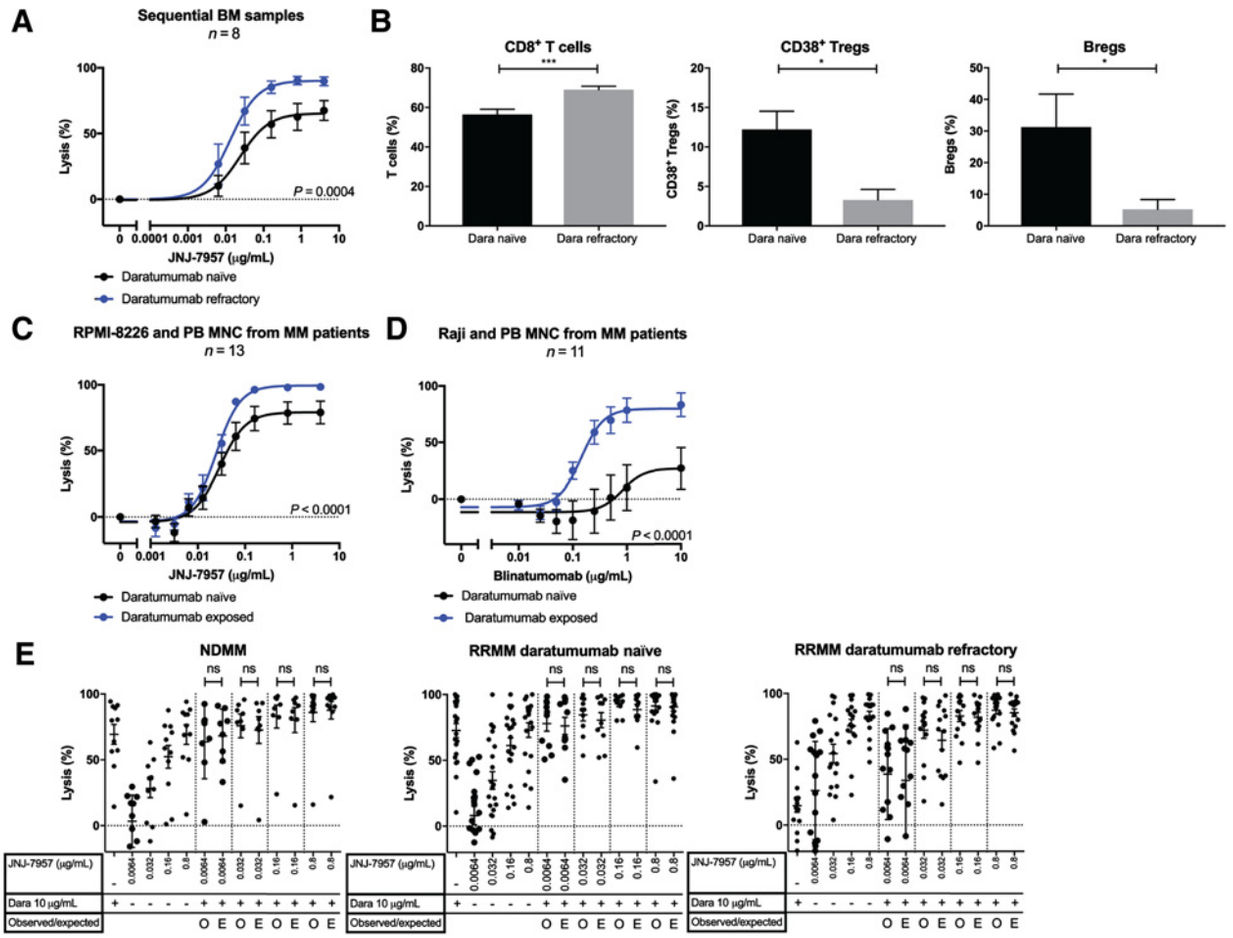

Figure 4.

Daratumumab treatment enhances JNJ-7957–mediated tumor cell lysis. **A,** JNJ-7957–mediated MM cell lysis in sequential BM samples obtained from 8 RRMM patients directly before the initiation of daratumumab (daratumumab-naïve, black) and at the time of refractory disease [progression during daratumumab treatment ($n = 4$) or failure to respond ($n = 4$); blue; median duration of treatment 3 months; range, 1–7 months]. BM-MNCs were incubated with JNJ-7957 (0.0064–4.0 μg/mL) for 48 hours, followed by flow-cytometric enumeration of surviving MM cells. Data represent mean ± SEM; experiments were performed in duplicate. *P* value between the indicated groups was calculated using nonlinear regression analysis. **B,** Frequency of CD8$^+$ T cells, CD38$^+$ Tregs, and Bregs in these 8 sequential BM samples, assessed by flow cytometry. Data represent mean ± SEM. *P* values were calculated using paired Student *t* test or Wilcoxon matched-pairs rank test; *, $P < 0.05$; ***, $P < 0.001$. **C,** JNJ-7957–mediated lysis of RPMI-8226 MM cell line, using sequential PB samples from 13 RRMM patients as effector cells (ratio of 10:1), which were obtained directly before the initiation of daratumumab treatment (black) and during daratumumab treatment (blue; median duration of treatment 7 months; range, 2–19 months). A BLI-based cytotoxicity assay was performed after a 48-hour incubation of RPMI-8226 cells with JNJ-7957 (0.00128–4.0 μg/mL) in the presence of these PB-MNCs. Data represent mean ± SEM; experiments were performed in duplicate. The statistical difference (*P* value) between the indicated groups was calculated using nonlinear regression analysis. **D,** Blinatumomab-mediated lysis of the Raji cell line, using sequential PB samples from 11 of the aforementioned 13 RRMM patients as effector cells (ratio of 10:1), which were obtained directly before initiation of daratumumab treatment (black) and during daratumumab treatment (blue; median duration of treatment 7 months; range, 2–14 months). A BLI-based cytotoxicity assay was performed after a 48-hour incubation of Raji cells with blinatumomab (0.01–10 μg/mL) in the presence of these PB-MNCs. Data represent mean ± SEM; experiments were performed in duplicate. The statistical significance (*P* value) between the indicated groups was calculated using nonlinear regression analysis. **E,** BM-MNCs obtained from NDMM ($n = 8$; median E:T ratio at baseline 1.14), daratumumab-naïve RRMM ($n = 10$; median E:T ratio at baseline 1.84), and daratumumab-refractory RRMM patients ($n = 14$; median E:T ratio at baseline 7.7) were treated with JNJ-7957 (0.0064–0.8 μg/mL) with or without daratumumab (10 μg/mL) for 48 hours, after which surviving CD138$^+$ MM cells were enumerated using flow cytometry. Dots represent individual data; error bars represent mean ± SEM; experiments were performed in duplicate. The observed tumor cell lysis (O) in samples treated with both daratumumab and JNJ-7957 was compared with the expected lysis (E), which was calculated as described in Materials and Methods. *P* values between observed and expected lysis were calculated using a paired Student *t* test. dara, daratumumab; HD, healthy donor; ns, not significant.

significantly enhanced by coincubation with daratumumab-exposed PB-MNCs, when compared with daratumumab-naïve PB-MNCs ($P < 0.0001$; **Fig. 4D**).

Direct combination of daratumumab and JNJ-7957 enhances MM cell lysis by JNJ-7957 in an additive fashion

To investigate whether the direct combination of daratumumab and JNJ-7957 enhanced tumor cell lysis, MM cell lines and BM-MNCs from patients were incubated with serial dilutions of JNJ-7957 with or without 10 μg/mL daratumumab for 48 hours. This daratumumab concentration was selected, because we previously showed that MM cell killing with 10 μg/mL daratumumab is associated with clinical response to daratumumab therapy (32). In these short-term experiments, daratumumab predominantly kills MM cells via its direct on-tumor mechanisms of action, whereas the development of immunomodulatory effects requires a longer period of time (∼8–12 weeks; refs. 21, 24, 38).

Short-term cotreatment with daratumumab-enhanced JNJ-7957 induced lysis of primary MM cells in both ND ($n = 8$) and daratumumab-naïve RRMM patients ($n = 10$) in an additive fashion (**Fig. 4E**). As expected, in daratumumab-refractory samples ($n = 14$), daratumumab did not have single-agent activity, and although JNJ-7957 was more active in these daratumumab-exposed samples, additional daratumumab did not further improve MM cell lysis. Because activated T cells upregulate CD38, we also assessed the impact of daratumumab on the frequency of $CD38^+$ T cells and on T-cell numbers in these coculture experiments. As expected, JNJ-7957 resulted in an increase in $CD38^+$ T cells, which was less pronounced in samples from daratumumab-refractory patients. Daratumumab partially abrogated the JNJ-7957–mediated increase in the frequency of $CD38^+$ T cells (Supplementary Fig. S8A). However, addition of daratumumab to JNJ-7957 had no or only modest effects on T-cell numbers (Supplementary Fig. S8B) and did not affect T-cell activation and degranulation (data not shown).

Additive effects were also observed with the $CD38^+$ RPMI-8226, UM9, and MM1.S MM cell lines. $CD38^-$ U266 cells were not killed by daratumumab, and JNJ-7957–mediated lysis was not further improved by the 48-hour coculture with daratumumab (Supplementary Fig. S9A). Similar results were obtained when UM9 cells were treated for 48, 72, and 96 hours with JNJ-7957 and daratumumab (0.01–10 μg/mL) at a low PB-MNC:MM cell ratio of 1:1 (Supplementary Fig. S9B).

Effects of soluble BCMA and APRIL on JNJ-7957 response

MM patients may have high levels of APRIL and sBCMA in serum, which could potentially interfere with the binding of JNJ-7957 to BCMA. We analyzed the effect of sBCMA and APRIL by treating the LUC-transduced RPMI-8226 cell line with JNJ-7957 in the presence of increasing concentrations of sBCMA or APRIL. sBCMA impaired the activity of JNJ-7957 in a dose-dependent manner (shift in EC_{50} values; **Fig. 5A**), but higher JNJ-7957 concentrations fully abrogated the negative effect of sBCMA. In contrast, only high concentrations of APRIL decreased the potency of JNJ-7957, but not the maximal killing of MM cells (**Fig. 5B**).

Next, we assessed sBCMA in the supernatants from BM-MNCs obtained from 14 RRMM patients. JNJ-7957 treatment for 48 hours resulted in a dose-dependent decrease in sBCMA levels (**Fig. 5C**). This indicates that sBCMA did not preclude effective killing of MM cells in our *ex vivo* assays.

JNJ-7957 effectively kills primary pPCL cells

Finally, we evaluated *ex vivo* activity of JNJ-7957 in BM samples from 6 patients with ND pPCL, which is characterized by an aggressive clinical behavior (39). JNJ-7957 was active in 5 of 6 samples, and tumor cell lysis in these pPCL samples was similar to lysis observed in NDMM and daratumumab-naïve RRMM samples, but lower than observed in daratumumab-refractory RRMM patient samples ($P = 0.0014$; **Fig. 6A**). Although the median E:T ratio in pPCL samples was approximately 8-fold lower, the extent of activation of both $CD4^+$ ($P = 0.0040$) and $CD8^+$ T cells ($P < 0.0001$), as well as the extent of degranulation of $CD8^+$ T cells ($P = 0.0141$), was superior in pPCL, when compared with NDMM (**Fig. 6B**). Degranulation of $CD4^+$ T cells was similar to that observed in NDMM.

Discussion

The current study shows that the BCMA×CD3 bispecific antibody JNJ-7957 has potent antitumor activity against MM cell lines in the presence and absence of BMSCs. In addition, in BM samples obtained from 55 patients, JNJ-7957 effectively eliminated malignant plasma cells in a dose-dependent manner, while sparing BCMA-negative NK cells and T cells. Importantly, these BM aspirates contain MM cells, as well as autologous effector T cells and immune suppressor cells. We also demonstrate that JNJ-7957–mediated killing of MM cells occurs via coupling of $CD4^+$ and $CD8^+$ T cells with BCMA-positive MM cells, which is followed by T-cell activation, secretion of inflammatory cytokines, FasL upregulation, and T-cell degranulation with release of cytolytic mediators such as granzyme B. Our findings are consistent with other studies showing that both $CD4^+$ and $CD8^+$ T cells contribute to tumor cell lysis induced by bispecific antibodies (9, 16, 40, 41).

The EC_{50} values of JNJ-7957 in NDMM or RRMM patients' samples varied from 0.017 to 0.054 μg/mL. These concentrations can likely be achieved in MM patients, because the serum concentration achieved at the highest dose safely tested in toxicity studies in cynomolgus monkeys was 1084.01 μg/mL (Cmax at day 22; Shoba Shetty and Suzette Girgis, Janssen Pharmaceuticals, personal communication, December 2, 2019). Similar anti-MM activity of JNJ-7957 was observed between patients with NDMM or daratumumab-naïve RRMM, which suggests that mechanisms of resistance toward prior therapies, such as IMiDs and PIs, do not affect the susceptibility of MM cells to JNJ-7957–dependent, T-cell-mediated lysis. Importantly, we observed enhanced killing capacity of JNJ-7957 in heavily pretreated patients who developed progression during daratumumab treatment, when compared with daratumumab-naïve patients. Similar findings were observed in serial BM samples obtained before initiation of daratumumab treatment and at the time of daratumumab-refractory disease. We also demonstrated that PB-MNCs from daratumumab-treated patients have an improved killing capacity in the presence of JNJ-7957, when compared with PB-MNCs obtained from daratumumab-naïve patients. This can be explained by our prior findings that daratumumab, next to its classic Fc-dependent immune effector mechanisms, also has immunomodulatory effects in the BM microenvironment including the elimination of $CD38^+$ immune suppressor cells, such as Tregs and Bregs, which results in increased T-cell numbers and T-cell activity (21–24, 26). Furthermore, the NADase activity of CD38 contributes to the development of T-cell exhaustion through reducing nicotinamide adenine dinucleotide (NAD^+) levels (42). In this respect, CD38-targeting antibodies

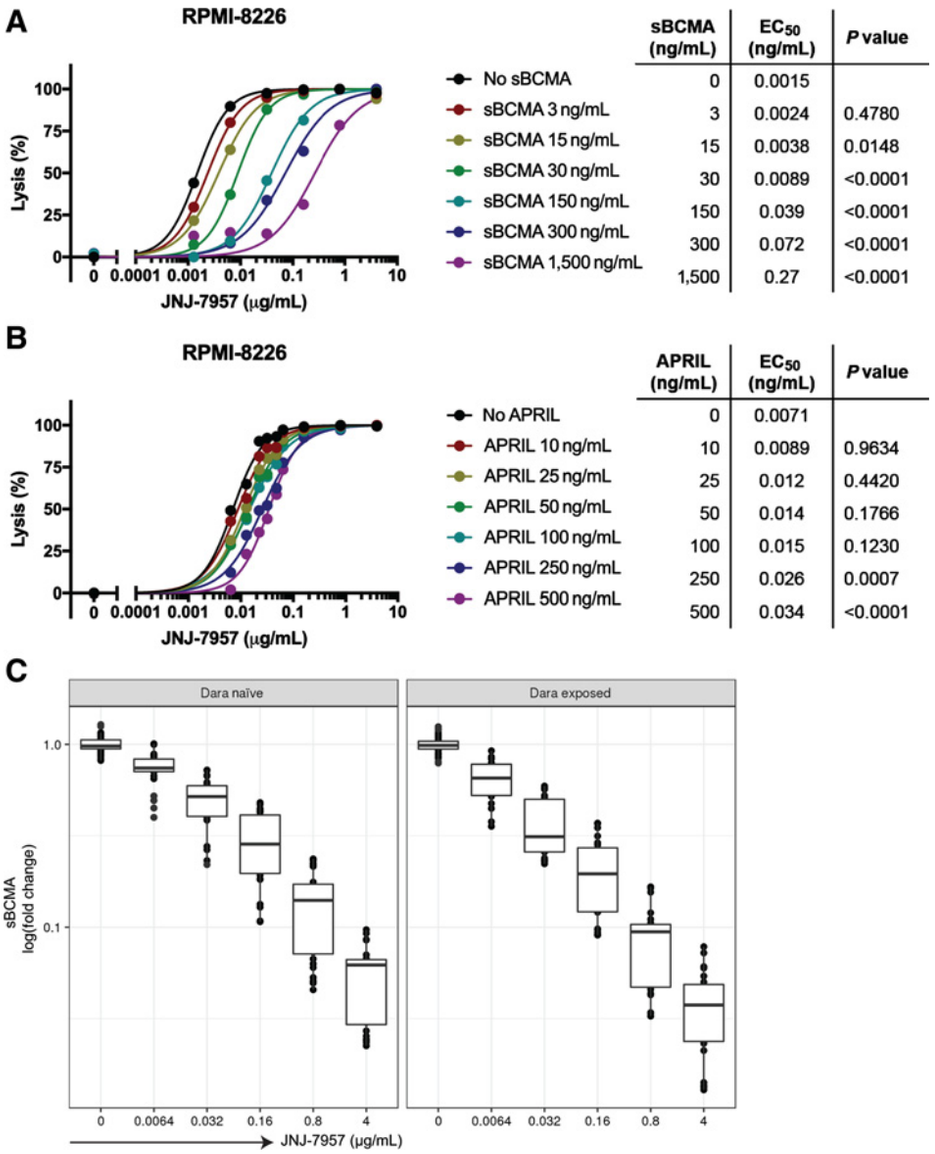

Figure 5.

Efficacy of JNJ-7957 is negatively affected by the presence of high concentrations of sBCMA or sAPRIL. **A,** The RPMI-8226 MM cell line was incubated with increasing concentrations of JNJ-7957 (0.00128–4.0 μg/mL) in the presence of healthy donor PB-MNCs (ratio of 10:1) with or without sBCMA (3–1,500 ng/mL) for 48 hours, followed by a BLI-based cytotoxicity assay. Data represent mean of 4 independent experiments performed in duplicate. EC_{50} values were compared using one-way ANOVA. **B,** The RPMI-8226 MM cell line was incubated with JNJ-7957 (0.0064–4.0 μg/mL) in the presence of healthy donor PB-MNCs (ratio of 10:1) with or without APRIL (10–500 ng/mL) for 48 hours, followed by a BLI-based cytotoxicity assay. Data represent mean of 4 independent experiments performed in duplicate. EC_{50} values were compared using one-way ANOVA. **C,** The level of sBCMA in the supernatant of BM-MNCs from RRMM patients (daratumumab-naïve $n = 7$; daratumumab-refractory $n = 7$) was assessed after a 48-hour incubation with JNJ-7957 (0.0064–4.0 μg/mL). Dots represent individual data; boxes represent first and third quartiles; the line represents the median. Data were normalized to solvent control-treated cells. EC_{50}, half maximal effective concentration.

improve antitumor T-cell immune response in mouse models by increasing NAD^+ levels in T cells (42). Next to these beneficial immunomodulatory effects, we showed in short-term coculture experiments that daratumumab also enhances JNJ-7957–mediated killing of MM cells in an additive fashion, probably by virtue of its direct on-tumor mechanisms of action, which are operational in short-term experiments (31, 32). We show that JNJ-7957 results in an increase in the frequency of $CD38^+$ T cells, which is partially abrogated by coculture with daratumumab. However, this had no negative impact on T-cell activity or T-cell numbers. Altogether, these data are in agreement with previous data showing that daratumumab, despite reducing CD38 expression on T cells, promotes T-cell expansion and increases their functional activity (21, 43). Altogether, our data indicate that the immunomodulatory and the direct antitumor effects of daratumumab work in concert with the T-cell–redirecting capacity of JNJ-7957, and this explains that prior daratumumab exposure was an independent determinant of *ex vivo* response to JNJ-7957. Importantly, although daratumumab-refractory MM cells were resistant to the direct effects of daratumumab, our results indicate that the immunomodulatory effects of daratumumab are preserved in daratumumab-refractory patients and contribute to immune-mediated killing of MM cells. Our data form the preclinical rationale for the evaluation of the combination of JNJ-7957 and daratumumab (TRIMM-2 study; NCT04108195), and indicate that prior or concomitant daratumumab treatment may also be beneficial for other T-cell–redirecting therapies such as blinatumomab.

We also evaluated the impact of several tumor- and host-related factors on sensitivity to JNJ-7957 therapy in all BM samples and separately in each patient group. The extent of *ex vivo* JNJ-7957 activity was dependent on T-cell frequency and the naturally occurring variation in E:T ratio with higher E:T ratios associated with improved MM cell lysis. However, similar to other BCMA×CD3 bispecific antibodies (9), marked JNJ-7957 activity was also evident at very low E:T ratios, in both cell lines and primary patient material such as pPCL samples, suggesting that T cells can serially lyse MM cells when redirected through JNJ-7957. Although the number of samples in

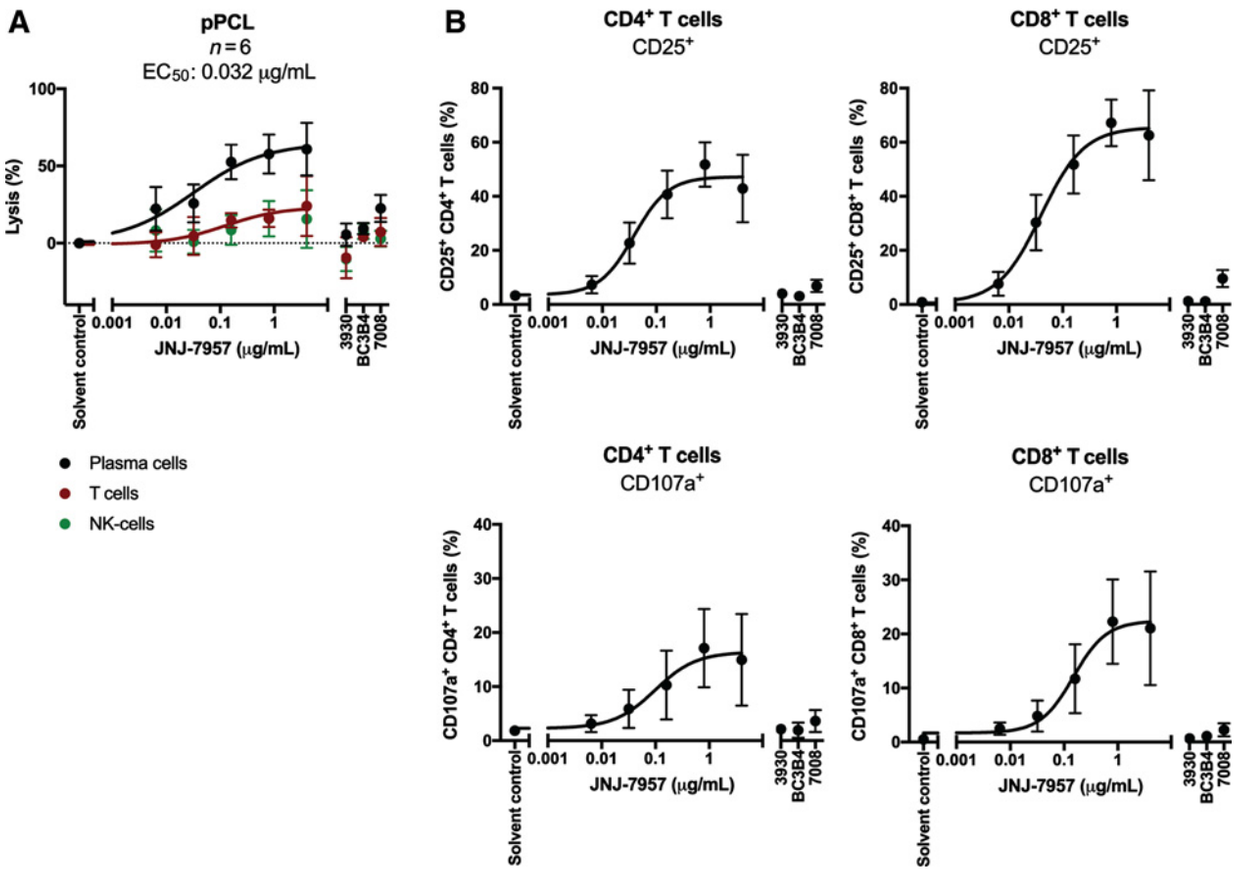

Figure 6.

JNJ-7957–mediated lysis of tumor cells in pPCL samples. **A,** BM-MNCs obtained from 6 pPCL patients were incubated with JNJ-7957 (0.0064–4.0 μg/mL) or control antibodies 3930, BC3B4, and 7008 (4.0 μg/mL) for 48 hours, after which the surviving $CD138^+$ tumor cells, as well as T and NK cells, were enumerated using flow cytometry analysis. Data represent mean ± SEM. All experiments were performed in duplicate. The EC_{50} value for plasma cell lysis is provided. **B,** JNJ-7957–mediated activation and degranulation of $CD4^+$ and $CD8^+$ T cells were assessed by flow-cytometric analysis of cell-surface expression of CD25 and CD107a, respectively. Data represent mean ± SEM. 3930, null×null control antibody; 7008, CD3×null control antibody; BC3B4, BCMA×null control antibody; EC_{50}, half maximal effective concentration.

each patient category was relatively small, our data suggest that various T-cell escape mechanisms have only a limited influence on the magnitude of redirected T-cell responses. The PD-1/PD-L1 axis negatively affected JNJ-7957 activity in daratumumab-naïve samples, suggesting that inhibitors of PD-1 and PD-L1 may improve the efficacy of JNJ-7957. However, the PD-1/PD-L1 axis had no significant effect on JNJ-7957 activity in daratumumab-refractory BM samples. We also observed a trend toward impaired JNJ-7957 efficacy in samples from RRMM patients with a higher frequency of Tregs, although this effect was completely abrogated by increasing the dose. Further studies will be required to elucidate whether other types of immunosuppressive cells, such as Bregs and MDSCs, or the subset of $CD38^+$ Tregs, affect JNJ-7957 activity.

In accordance with other reports (9, 11, 44), we demonstrate that BCMA cell-surface expression is variable with an increase of BCMA expression at the time of relapse, when compared with diagnosis. In addition, BCMA expression was not affected by daratumumab, whereas daratumumab has been shown to reduce cell-surface expression of CD38 and some other cell-surface proteins such as CD56 and CD138 (32, 43). Importantly, the efficacy of JNJ-7957 to kill MM cells was not affected by extent of BCMA expression, as observed in our sample set. Similarly, *ex vivo* sensitivity to the BCMA-T-cell bispecific antibody EM801 was also independent of BCMA expression levels (9). Preliminary evidence indicates that BCMA CAR T-cell efficacy is also independent of BCMA expression levels (45, 46). This suggests that even limited amounts of BCMA allow efficient immune synapse formation with T cells.

When compared with healthy individuals, MM patients have higher serum levels of sBCMA and APRIL (median serum sBCMA level approximately 100-150 ng/mL (11, 47, 48); median serum APRIL level approximately 2.7 ng/mL (49)). A common concern raised with BCMA-targeting therapies, is whether sBCMA impairs efficacy by acting as a drug sink. We and others showed that sBCMA reduces the potency of BCMA×CD3 bispecific antibodies and BiTEs, but the maximal lysis is not affected (16). The sBCMA sink may partly explain why the clinically active dose of the anti-BCMA BiTE AMG420 is substantially higher than that for blinatumomab (17, 37). In contrast, the addition of APRIL had minimal impact on the potency of JNJ-7957. Only high levels of APRIL (≥250 ng/mL) interfered with JNJ-7957–mediated lysis of MM cells, possibly by interfering with the binding of JNJ-7957 to BCMA, or via the activation of survival signaling pathways.

Currently, several BCMA-based T-cell redirection therapies are in clinical development in MM. Although BCMA-specific CAR T cells are promising, this T-cell redirection therapy requires *ex vivo* engineering and expansion of patient-specific T cells, which may form a barrier to universal access in the near future. In contrast, T-cell bispecific antibodies or BiTEs are "off-the-shelf" products and, in principle, applicable to almost all patients (50).

In summary, in the present study, we demonstrate that JNJ-7957 redirects T cells to BCMA-expressing MM cells, which leads to marked activity against MM cell lines and primary MM cells from patients with NDMM, as well as heavily pretreated RRMM. This suggests lack of cross-resistance between the different classes of anti-MM drugs and JNJ-7957. Furthermore, killing capacity was independent of BCMA expression levels and only moderately affected by the immune suppressive BM microenvironment. Our data form the preclinical rationale for the ongoing phase I study of JNJ-7957 (NCT03145181) in heavily pretreated MM patients. In addition, our data in samples from daratumumab-exposed patients suggest that clinical evaluation of the combination of daratumumab and JNJ-7957 may be warranted based on the potential for a combination of effects, including a more permissive microenvironment for JNJ-7957 function.

Disclosure of Potential Conflicts of Interest

J. Sendecki is an employee/paid consultant for Janssen. F. Gaudet is an employee/paid consultant for and holds ownership interest (including patents) in Janssen. S. Zweegman is an advisory board member/unpaid consultant for Janssen, Celgene, and Takeda. H.C. Adams is an employee/paid consultant for Janssen R&D, LLC. T. Mutis reports receiving commercial research grants from Takeda, Janssen and Genmab. N.W.C.J. van de Donk reports receiving commercial research grants from Janssen Research and Development, Celgene, Amgen, Novartis, BMS, is an advisory board member/unpaid consultant for Janssen Research and Development, Amgen, Celgene, BMS, Takeda, Roche, Bayer, Servier, and Novartis. No potential conflicts of interest were disclosed by the other authors.

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