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SHORT REPORT

Identifying clinical response to daratumumab therapy in relapsed/refractory multiple myeloma using a patient-derived in vitro model

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Abstract

Response to daratumumab in patients with relapsed/refractory multiple myeloma is heterogeneous, and a reliable biomarker of response is lacking. We aimed to develop a method that identifies response to daratumumab therapy. Patient-derived MM cells were collected before start of daratumumab treatment and were cultured in a hydrogel-based culture system. The extent of antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity in vitro was associated with both clinical response and progression-free survival in corresponding patients. Together, our results demonstrate that in vitro sensitivity to daratumumab therapy in a hydrogel culture with primary MM cells might be used to identify patients most likely to benefit from treatment.

KEYWORDS
daratumumab, multiple myeloma, patient-derived plasma cells, relapsed/refractory multiple myeloma, response to therapy

1 | INTRODUCTION

The introduction of anti-CD38 monoclonal IgG1-κ antibody daratumumab has considerably changed the treatment landscape of multiple myeloma (MM) over the past decade [1]. However, response to daratumumab is heterogeneous and unpredictable, especially in the relapsed/refractory MM (RRMM) setting. The overall response rate was 31%–36% in early-phase clinical trials testing daratumumab as monotherapy in RRMM [2, 3]. While CD38 expression on MM cells was shown to be associated with daratumumab responses, it is considered too heterogeneous to be used in clinical practice [4, 5]. A robust and reliable biomarker of response to daratumumab is currently lacking.

In recent years, patient-derived organoids from solid tumors have demonstrated great potential in predicting cancer treatment responses [6]. In hematological malignancies and MM specifically, personalized medicine is hindered by the limited life-span of primary malignant cells in vitro [7, 8]. Additionally, testing the efficacy of monoclonal antibodies is less straightforward than conventional chemotherapy or targeted therapy, since there is a need for effector cells or proteins. Multiple efforts have been made to predict response to therapy in RRMM, both by using clinical and/or genetic features, and by measuring in vitro drug sensitivity [7–12]. So far, however, none of these attempts have been implemented into clinical practice. In the current study, we explored the ability of an in vitro culture model to identify responses to daratumumab treatment in patients with RRMM.

2 | METHODS

Clinical data, CD38 mean fluorescent intensity (MFI) and bone marrow mononuclear cells (BMNCs) were obtained from patients with RRMM that had been scheduled to start daratumumab after bone marrow aspiration. This study was approved by the local ethics committee of the Utrecht University Medical Center and was conducted in accordance with the Declaration of Helsinki. All included patients provided written informed consent.

Patient-derived samples were cultured as described previously [13]. BMNCs were thawed and resuspended in RPMI 1640 GlutaMAX HEPES culture medium (Life Technologies), supplemented with 10% heat-inactivated fetal bovine serum (Biowest), and 100μg/mL penicillin-streptomycin (Gibco/Life Technologies). MM samples were then transferred to 96-well plates containing 100μl 0.5% PuraMatrix hydrogel (Corning) per well. Samples were plated at 2×10^5 BMNCs per well and supplemented with 100ng/ml IL-6 (PeproTech) and 100ng/ml APRIL (R&D Systems). Samples were kept at 37°C and 5% CO2 for 6 days until daratumumab treatment.

After incubation for 1 h with either 0.1 μg/mL daratumumab or 0.1 μg/mL IgG1κ isotype control (Invitrogen), complement-dependent cytotoxicity (CDC) was induced by adding 10% non-heat inactivated pooled human serum from healthy donors. Antibody-dependent cellular cytotoxicity (ADCC) was induced by adding healthy-donor peripheral blood mononuclear cells (PBMC) in a 10:1 effector-to-target ratio, calculated based on the percentage of plasma cells within the BMNCs. PBMCs from healthy donors were obtained by blood withdrawal from volunteers aged 18–65 years working at our local institution. All donors provided written informed consent. PBMC were isolated by Ficoll (GE Healthcare) separation and either used directly or cryopreserved in 10% dimethyl sulfoxide (Honeywell) for later use. Daudi cells, which have a proven high sensitivity to daratumumab, were treated similar to the primary MM cells and served as a positive control [14].

After 24 h, BMNCs were retrieved from the gel and living MM cells were distinguished with flow cytometry (LSR Fortessa, BD Biosciences) using anti-CD138-PE (Beckman Coulter), multi-epitope anti-CD38-FITC (Cytognos) and TO-PRO-3 (Thermo Fisher Scientific). In the case of ADCC assays, PBMCs were labeled with CellTrace Violet (Invitrogen). Flow-Count Fluorospheres (Beckman Coulter) were used to calculate absolute numbers of surviving cells, and the data were analyzed with FlowJo software (BD). Representative flow cytometry plots are displayed in Figure S1. Statistical analyses and preparation of figures were performed using statistical programming software R in Rstudio (version 4.0.0) and GraphPad Prism (version 10.0.2).

3 | RESULTS AND DISCUSSION

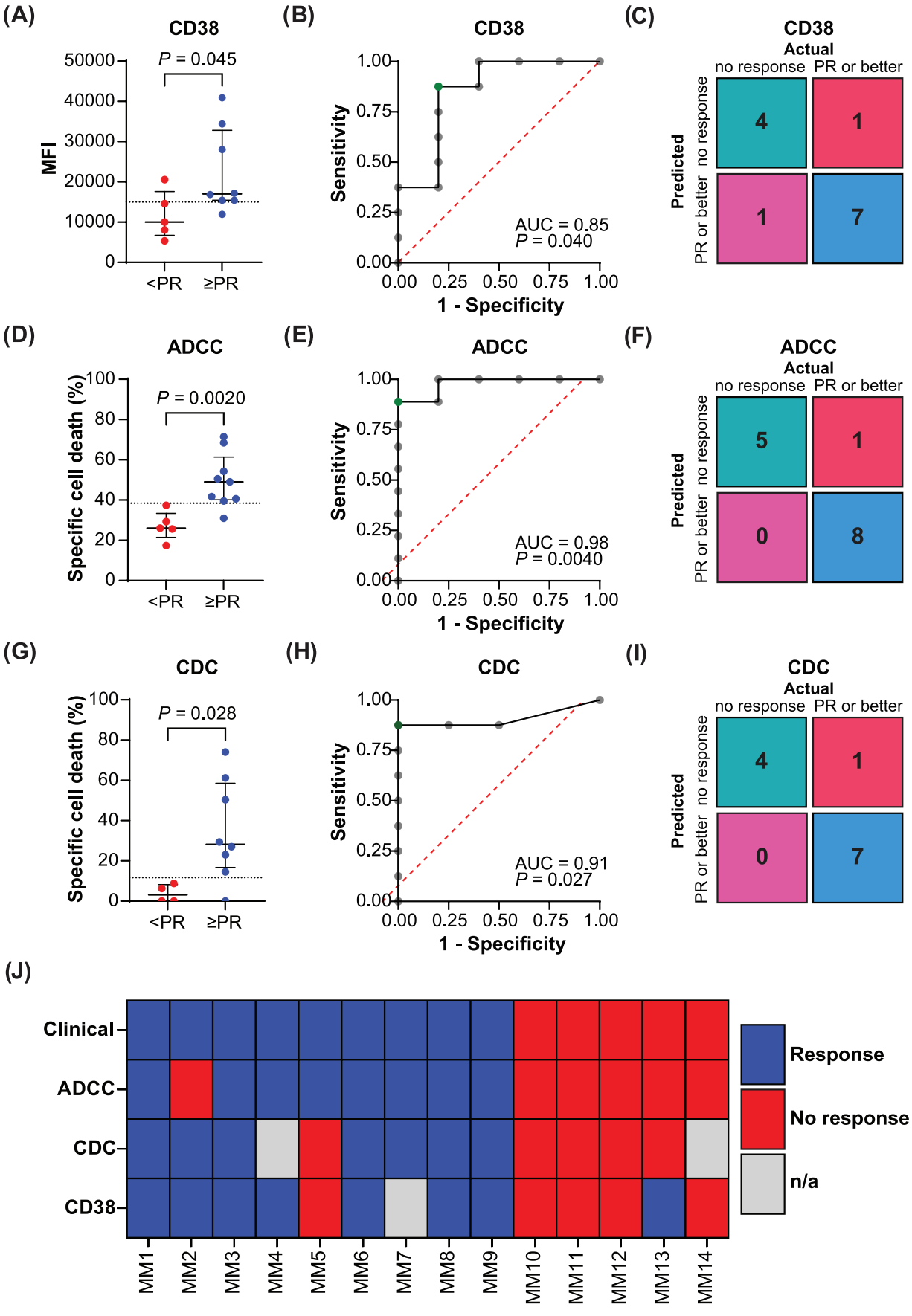
BMNCs were obtained from 14 patients that were going to be treated with daratumumab either as monotherapy ($n = 5$) or as part of a combination regimen ($n = 9$), most often together with lenalidomide and dexamethasone (Table S1). Daratumumab was given as 2nd until 5th line of therapy and treatment duration ranged from 4 to 119 weeks and ongoing. Nine patients obtained a best response of at least a partial response (PR), while five patients did not achieve an objective response.

Expression of CD38 would be a straightforward biomarker of daratumumab response, but earlier studies have demonstrated its heterogeneity. Therefore, we compared CD38 MFI with ADCC and CDC in their ability to predict response to therapy. Corroborating earlier studies, we found a significant difference in mean CD38 MFI between patients with a clinical response and those without a response ($p = 0.045$) (Figure 1A, Figure S2A). An ROC curve was generated to appreciate the discriminative ability of the results, which revealed an area under the curve (AUC) of 0.85 (95% confidence interval (CI) 0.61–1.00, $p = 0.040$) (Figure 1B). Next, by using Youden's index, the optimal cut-off value of CD38 MFI was established to be 15015 MFI. Using this cut-off value to identify response to daratumumab resulted in correct classification of 11 of 13 evaluable patients (Figure 1C).

In vitro ADCC-induced MM cell death ranged from 17.4% to 71.4% (Figure S2B). We found that ADCC was strongly associated with the clinical response by the corresponding patients with a median ADCC of 49.1% for patients who achieved a partial response (PR) or better and 26.0% for patients who did not ($p = 0.0020$) (Figure 1D). The ROC curve revealed an AUC of 0.98 (95% CI 0.91–1.00, $p = 0.0040$) (Figure 1E), with an optimal cut-off value of 38.5% ADCC. Using this cut-off value, clinical responses could be correctly classified for 13 of 14 patients (Figure 1F).

For 12 out of 14 patients, we also tested the ability of CDC to classify clinical response to daratumumab. Eight primary samples were derived from patients that achieved at least a PR upon daratumumab treatment, while four were derived from patients who did not. In vitro results of these assays were more heterogeneous than ADCC, with a range of 0% to 92.8% CDC (Figure S2C). There was a significant association between CDC results and the clinical response to daratumumab by the corresponding patients ($p = 0.028$) (Figure 1G). Median CDC for patients who achieved a PR was 28.2% versus 3.2% for patients who did not achieve a PR. The resultant ROC curve had an AUC of 0.91 (95% CI 0.72–1.00, $p = 0.027$) (Figure 1H), and the subsequent optimal cut-off value of CDC was 11.7%. Using this cut-off value to discriminate clinical responders from nonresponders resulted in an accurate classification of 11 of 12 patients (Figure 1I). Figure 1J summarizes the clinical response to daratumumab by each patient and the classification of response by ADCC, CDC, and CD38 expression using their respective optimal cut-off values.

Additionally, we examined the association between CD38 expression, ADCC or CDC and the clinical progression-free survival (PFS) of daratumumab treatment. Patients were divided into two groups based on the optimal cut-off values as established by the ROC curves. There was no significantly longer PFS in the group of patients whose CD38 expression was above the cut-off value of 15015 MFI, although a trend was observed ($p = 0.067$) (Figure 2A). Notably, the patients whose in vitro ADCC was above the cut-off value of 38.5% cell death or those with CDC above 11.7% cell death experienced a significantly longer PFS clinically (ADCC, $p = 0.0097$; CDC, $p = 0.012$) (Figure 2B,C).



The results of the current study illustrate that it is possible to assess the efficacy of monoclonal antibodies in vitro prior to initiating treatment in patients with RRMM. We observed significant differences in ADCC and CDC between patients that achieved a PR versus patients that did not respond. Additionally, we assessed the relation between the in vitro results and the PFS of daratumumab-based treatment. We demonstrated that ADCC and CDC, but not CD38, correlate with PFS in RRMM patients treated with daratumumab.

Our study was performed in an in vitro culture model in which patient-derived malignant cells were co-cultured with healthy-donor PBMCs as immune effector cells for ADCC or healthy-donor serum for CDC [13]. Allogeneic effector cells and proteins were used to ensure the robustness and reproducibility of our assays. We did not find a difference in the efficacy of daratumumab-induced ADCC comparing autologous and allogeneic PBMCs in a small cohort of patient-derived MM cells (Figure S3). Resistance to antibody-induced destruction by complement in tumor cells is primarily induced through the expression and secretion of complement-regulatory proteins (CRPs) [15, 16]. In MM patients that were treated with daratumumab, expression of CRPs CD55 and CD59 was significantly increased upon progression of disease [4]. However, little is known about the existence of tumor cell-extrinsic mechanisms of resistance to complement-mediated killing. Combined, our data indicate that the use of allogeneic PBMCs or serum recreates an in vitro platform able to identify clinical response to daratumumab.

Importantly, both ADCC and CDC were shown to play a role in killing patient-derived MM cells during development of daratumumab [1, 14, 17]. Although daratumumab reduces the number of NK cells, themselves important effectors of ADCC, previous research found no association between response rates and degree of NK cell reduction after daratumumab treatment [18]. Notably, surviving NK cells retained their capacity for ADCC. However, it has been revealed that working mechanisms extend beyond ADCC and CDC. Antibody-dependent cellular phagocytosis by monocytes and macrophages was shown to contribute to the killing mechanism of daratumumab [1, 9, 17]. Also, it has been demonstrated that daratumumab not only operates via classic Fc-receptor-dependent effector functions, but also exerts immunomodulatory effects. Daratumumab therapy was shown to improve T-cell mediated killing by depleting CD38-positive immune suppressive regulatory T cells, regulatory B cells and myeloid derived suppressor cells [1, 17]. The relevance of these mechanisms in the context of a further optimized predictive model remains to be investigated.

A limitation of this study is the size and heterogeneity of the current in vitro cohort, reflecting the exploratory nature of this study. Still, CDC and ADCC outcomes were significantly different between clinical responders and nonresponders, without a high degree of overlap. Several patients in our cohort received daratumumab as part of a combination regimen, where a PR might have been achieved due to antimyeloma activity by one of the other drugs instead of daratumumab itself. However, the clinical responses to daratumumab combination therapy align accurately with the in vitro response to daratumumab-induced ADCC or CDC, regardless of the received treatment regimens.

Ultimately, the goal of our study is to develop an in vitro drug sensitivity method to inform clinicians on choice of therapy in patients with relapsed MM. Using the current method, it would take 1 week from obtaining a bone marrow aspirate until daratumumab sensitivity can be determined. However, we believe that our assay can be shortened by decreasing the incubation time with BMNCs prior to the addition of daratumumab and by limiting ADCC and CDC measurements from 24 h to several hours. These changes would considerably increase the usability of ex vivo drug screening in clinical management.

The cut-off values of CD38, ADCC, and CDC that we determined above remain to be validated in a separate validation cohort. A caveat is the possibility of incorrectly classifying patients with a good clinical

FIGURE 1 CD38 mean fluorescent intensity (MFI), antibody-dependent cellular cytotoxicity (ADCC), and complement-dependent cytotoxicity (CDC) associate with clinical response to daratumumab therapy. (A) Comparison of CD38 mean fluorescent intensity (MFI) in plasma cells from patients with relapsed/refractory multiple myeloma (RRMM) that either did not respond clinically (<partial response [PR], $n = 5$) or that did respond clinically (≥PR, $n = 8$). Dots denote individual patient samples, while the dashed line (MFI 15015) corresponds with the optimal cut-off value as determined by the Youden's index in (B). Statistical difference between these groups was calculated using a two-tailed Mann–Whitney test. Horizontal bar represents the median and error bars indicate the interquartile range. **(B)** Receiver operating characteristics (ROC) curve of data in (A). The dashed line represents an AUC_{ROC} of 0.5 and indicates no predictive value. Green dot identifies the Youden's index. **(C)** Confusion matrix comparing clinical responses of patients with predicted responses based on the cut-off value of a CD38 MFI of 15,015, as established in (B). **(D)** Results of in vitro ADCC assays. RRMM patient-derived bone marrow mononuclear cells were treated with daratumumab or isotype control and incubated for 24 h with healthy-donor PBMCs at an effector-to-target ratio of 10:1. Samples were derived from nine patients that achieved a clinical response and five patients that did not achieve a measurable response. ADCC was calculated on the absolute number of surviving CD138+CD38+ plasma cells in the treated versus isotype conditions, as measured by flow cytometry. Dotted line (38.5% cell death) corresponds with the optimal cut-off value. **(E)** ROC curve of ADCC results in (D). Method similar to (B). **(F)** Confusion matrix comparing clinical responses of patients with predicted responses based on the cut-off value of 38.5% specific cell death after ADCC, as established in (E). **(G)** Results of in vitro CDC assays, similar to (D), with RRMM patients that did not obtain a PR ($n = 4$) and patients that obtained a PR or better ($n = 8$). CDC was measured by adding pooled healthy-donor non-heat inactivated serum. The dotted line (11.7% cell death) corresponds with the optimal cut-off value. **(H)** ROC curve of CDC data in (G). Method similar to (B) and (E). **(I)** Confusion matrix comparing clinical responses of patients with predicted responses based on the cut-off value of 11.7% specific cell death after CDC, as established in (H). **(J)** Comparison of the clinical response of each included patient with the classification of the response according to ADCC, CDC, and CD38 MFI. Each column represents an individual patient. Blue color denotes a PR for the clinical response or a positive response prediction for each of the respective tests, while red denotes a negative response. Grey square indicates no test result available.

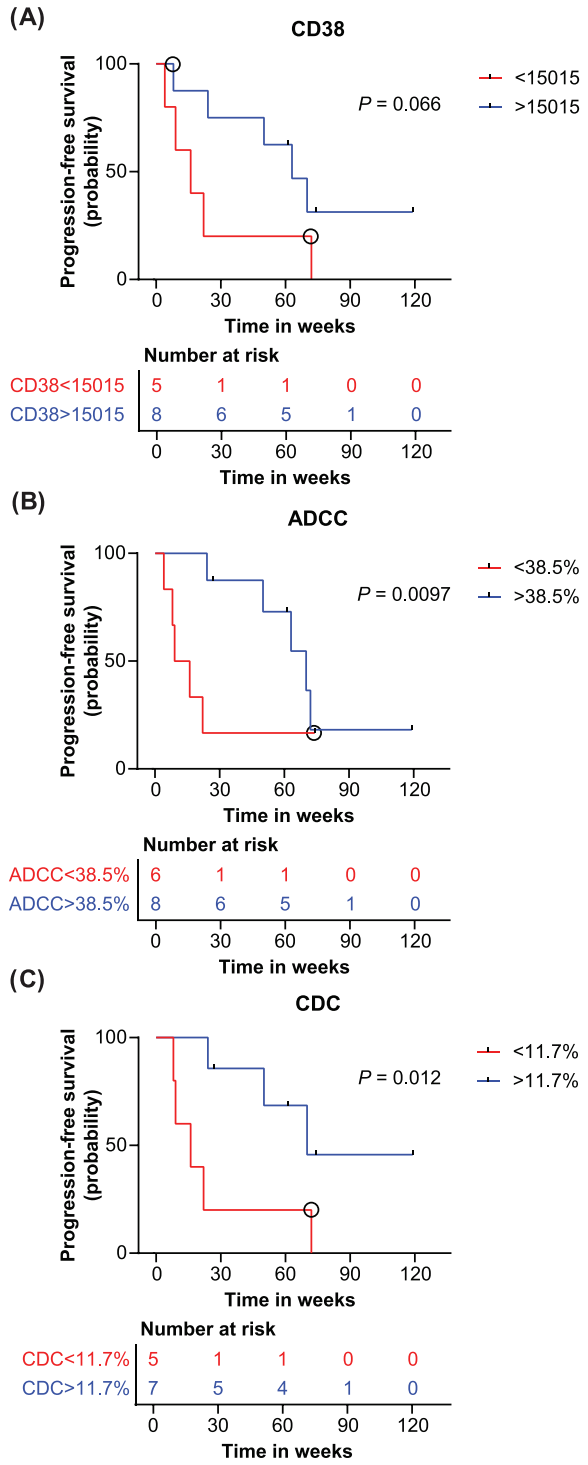


FIGURE 2 **Progression-free survival is higher in patients with antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) above the cut-off value.** **(A)** Kaplan–Meier curve of the progression-free survival (PFS) of daratumumab treatment, comparing patients with CD38 mean fluorescent intensity (MFI) above and below the cut-off value of 15015 MFI. Statistical difference between groups was calculated with the Wilcoxon Test. Incorrectly classified patient samples are marked with a circle. Sample MM13 was incorrectly classified as a response, with CD38 expression above the cut-off value. Sample MM5 was incorrectly classified as a non-responder, with CD38 expression below the cut-of value. **(B)** Comparison of PFS of relapsed/refractory multiple myeloma (RRMM) patients with in vitro ADCC above or below cut-off value of 38.5% cell death as established previously. Incorrectly classified patient samples are marked with a circle. Sample MM2 was incorrectly classified as a non-responder, as in vitro ADCC was below 38.5% **(C)** PFS of RRMM patients of which in vitro CDC was above or below cut-off value of 11.7% cell death, as established previously. Incorrectly classified patient samples are marked with a circle. Sample MM5 was incorrectly classified as a non-responder based on an in vitro CDC below 11.7%.

response but a low level of ADCC or CDC in vitro. To be clinically useful, a predictive model of therapeutic response should prevent ineffective treatment of patients who are not likely to benefit, while simultaneously not withhold effective therapy from patients who will respond. The significant differences that we observed for both ADCC and CDC in our current cohort suggest incorrect classification should be a rare event, but this remains to be validated in a larger cohort.

We explored an in vitro method to identify response to daratumumab treatment in patients with RRMM. We found that high ADCC and CDC in patient-derived MM cells within a hydrogel-based culture system has a strong positive correlation with clinical response and PFS. Our data pave the way for in vitro drug sensitivity screens using primary malignant cells as a clinical decision aid to determine whether a patient is likely to benefit from treatment.

AUTHOR CONTRIBUTIONS

N.v.N., V.P., and M.C.M. designed the research. N.v.N., M.C., and L.A. performed experiments and/or collected data. N.v.N., M.C., V.P. M.J., and M.C.M. analyzed and interpreted data. N.v.N. performed statistical analyses. N.v.N. wrote the initial draft. M.C., M.J., V.P., and M.C.M. revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

M.C.M received honoraria paid to institution by Jansen Cilag, BMS, Gilead, Medscape, GSK, and Alnylam. M.J. received research funding by Novartis. V.P. received royalty payments related to venetoclax. The other authors declare no potential conflict of interest.

FUNDING INFORMATION

Spafima Foundation

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the local ethics committee of the Utrecht University Medical Center and was conducted in accordance with the Declaration of Helsinki.

PATIENT CONSENT STATEMENT

All included patients provided written informed consent.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

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SUPPORTING INFORMATION

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

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