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Research paper

In vitro testing of drug response in primary multiple myeloma cells using a microwell-based technology

Josefine Krüger [a,*], Igor Wolfgang Blau [b], Olga Blau [b], Alice Bettelli [c], Laura Rocchi [c], Massimo Bocchi [c], Jan Krönke [a,d], Lars Bullinger [b,d], Ulrich Keller [a,d,e], Axel Nogai [a]

[a] *Department of Hematology, Oncology and Cancer Immunology, Campus Benjamin Franklin, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin 12203, Germany*
[b] *Department of Hematology, Oncology and Cancer Immunology, Campus Virchow Klinikum, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin 13353, Germany*
[c] *Cellply S.r.l., Bologna 40138, Italy*
[d] *German Cancer Consortium (DKTK), partner site Berlin, a partnership between DKFZ and Charité-Universitätsmedizin Berlin, Berlin 12203, Germany*
[e] *Max-Delbrück-Center for Molecular Medicine, Berlin 13125, Germany*

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ABSTRACT

Multiple myeloma is an aggressive neoplasm of plasma cells. While numerous drugs have gained approval, the absence of established predictive markers for individual drug responses poses a challenge. In this study, we explored the microwell- and fluorescence-based Cellply CC-Array® technology for high-throughput analysis of *in vitro* drug responses as a potential predictive marker for patient treatment outcomes. Furthermore, we investigated its application for evaluating effector cell effectiveness. Mononuclear cells were isolated from the bone marrow of 22 patients, and *in vitro* drug response of primary myeloma cells was analyzed. *In vitro* responses towards melphalan, bortezomib, and dexamethasone in primary patient samples correlated with clinical response of the patients. The approach exhibited limitations in identifying sensitivity towards lenalidomide, daratumumab, and elotuzumab due to limited culturing time caused by poor myeloma viability *in vitro*. Through the analysis of cell proximity, the platform enabled the assessment of individual anti-tumor activity from NK and T cells. In summary, the CC-Array microwell technology allowed assessment of myeloma cell responses to selected drugs used in multiple myeloma therapy *in vitro*. To further validate these *in vitro* results against *in vivo* outcomes, screening a larger cohort is necessary.

1. Introduction

Multiple myeloma (MM) is an aggressive malignancy characterized by proliferation of monoclonal plasma cells and organ damage [1]. Various drug classes, such as proteasome inhibitors, immunomodulatory derivatives (IMiDs), cytostatic drugs, corticosteroids, monoclonal antibodies (mAb), bispecific antibodies, and CAR-T cells, are approved for MM treatment. Despite the availability of highly effective drugs, MM remains incurable, with a median overall survival of approximately 10 years [2].

The approval of numerous drug combinations poses a challenge in treatment decisions due to the lack of established predictive markers. Myeloma cells frequently demonstrate resistance to various drugs, resulting in the use of ineffective substances and unnecessary side effects. A personalized medicine approach could enhance treatment response, prolong overall survival, and improve quality of life[3].

In vitro drug testing platforms offer a method to potentially determine the most effective therapy for individual patients. Previous research demonstrated the applicability of approaches such as the FACS-based platform My-DST, achieving a sensitivity of 96 % and specificity of 88 % in predicting drug responses compared to the *in vivo* outcomes in 55 MM patients [4]. A pharmacoscopy-based platform, allowing high-throughput *ex vivo* response prediction through apoptosis detection based on cell morphology, also showed promise [5]. While several promising *in vitro* drug testing approaches for MM have been proposed, no routine-use technology has been established.

The recently developed VivaCyte platform, utilizing a CC-Array microfluidic device and image-based analysis through fluorescence

* Corresponding author.
E-mail address: josefine.krueger@charite.de (J. Krüger).

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detection of tumor and other effector cells, demonstrated precise prediction of responders and non-responders in patients with acute myeloid leukemia [6]. Unlike most other platforms, this platform can detect co-localization and cell-cell contact of tumor cells with other cells, including NK cells, T cells, and bone marrow mesenchymal stromal cells (BMMSC). This is crucial as almost all myeloma drugs are influenced or dependent on these effector cells, and the development of resistance is likely partially driven by them [7–16]. Additionally, with the increasing role of new T cell-based therapies, T cell fitness becomes relevant as a known predictive marker for therapy response [17].The VivaCyte platform was utilized in a Beta version to demonstrate antibody-dependent cellular cytotoxicity (ADCC), a primary mechanism of action of monoclonal antibodies, when treating myeloma cells with daratumumab *in vitro* [18].

In this study, we investigated a novel microwell-based technology as a strategy to determine *in vitro* drug responses in primary MM cells. Assessing drug responses with this platform identified *in vitro* sensitivities and resistances from different patients to the substances bortezomib, dexamethasone, and melphalan in concordance with the patients' clinical courses, but showed limitations for lenalidomide, daratumumab, and elotuzumab. The platform also demonstrated the ability to determine anti-tumor activity of effector cells as NK and T cells, an important predictive marker for response to new cell-based therapies.

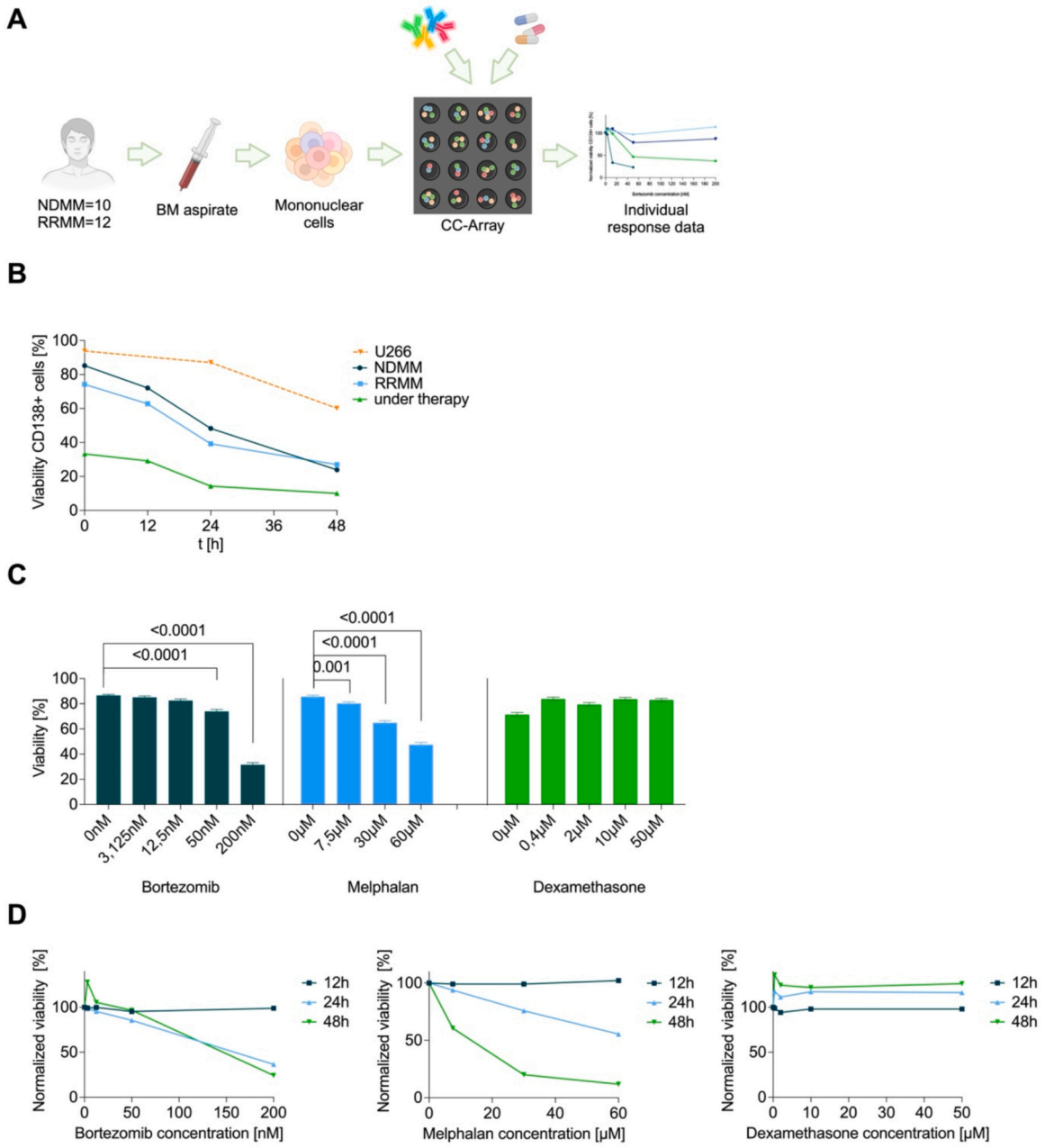

Fig. 1. In vitro drug testing using the Cellply VivaCyte platform. A. Process of in vitro drug testing with the VivaCyte platform. B. Absolut viability of U266 cell line and all primary myeloma cell samples in negative control (DMSO) divided by NDMM (n=10), RRMM (n=7) and under therapy (n=3). C. Absolut viability of U266 at timepoint 24 h of bortezomib, melphalan and dexamethasone in different concentrations. Kruskal-Wallis-Test with Dunn's multiple comparisons. D. Dose-response curves of U266 for bortezomib, melphalan and dexamethasone with normalized viability after 12 h, 24 h and 48 h.

2. Material and methods

2.1. Cell culture

To establish testing conditions and parameters, the human MM cell line U266 was used. The cells were cultured in RPMI-1640 (Thermo Fisher Scientific) supplemented with 10 % fetal bovine serum (PAN-Biotech GmbH) and 1 % penicillin/streptomycin (Thermo Fisher Scientific). Cells were maintained at 37°C and 5 % CO_2 in a humidified incubator. Cell passage was conducted twice a week, and cells were resuspended at the concentration of 2×10^5 cells/ml.

2.2. Primary patient samples

EDTA bone marrow samples were obtained from 22 patients with newly diagnosed (NDMM) and relapsed or refractory MM (RRMM) at Charité Universitätsmedizin Berlin. All patients signed a written informed consent form, and approval from the ethics committee was obtained (EA1/152/10, EA2/142/20). To ensure good cell viability, all samples were processed on the same day as collection. Mononuclear cells (MNC) were isolated by Ficoll (Biochrom GmbH). Lysis (Qiagen) was performed up to two times if necessary. Finally, the cells were suspended in RPMI-1640 supplemented with 20 % fetal bovine serum and 1 % penicillin/streptomycin.

2.3. Fluorescence antibodies

All cells were stained with Calcein AM (Invitrogen™) or CellTracker™ Blue CMAC (Invitrogen™) depending on the other fluorochromes used in each experiment. For evaluating cell viability, propidium iodide (PI; Invitrogen™) was used. To mark myeloma cells, we utilized an anti-CD138 antibody (Alexa Fluor® 647, Bio-Rad Laboratories Ltd.). For NK cells, anti-CD16 and anti-CD56 (both BV421, BD Horizon™), for T cells anti-CD3 (Alexa Fluor® 488, BioLegend) was used.

2.4. VivaCyte drug testing platform

An overview of the drug testing process is shown in Fig. 1A. To evaluate *in vitro* drug responses, the Cellply VivaCyte platform (Cellply S.r.l.) was utilized in a Beta version. Using image-based fluorescence detection of different cell types and cell viability, this platform allows testing up to 16 different conditions simultaneously, as previously described [18,19]. All preparation steps, such as cell staining, washing, and suspension in different concentrations of the drugs, can be automated by the instrument. Testing takes place in a CC-Array microfluidic device containing 16 channels, each with 1200 microwells, which can be analyzed individually. In our experiments, the time points 0 h, 12 h, 24 h, and 48 h were used for the analysis. We tested representatives of all major substance classes, including lenalidomide, bortezomib, melphalan, dexamethasone (all from Sigma-Aldrich), elotuzumab (Bristol Myers Squibb™), and daratumumab (Selleck Chemicals) at different concentrations, respectively. DMSO (AppliChem GmbH) was used as the negative control, and 10 mM H_2O_2 (Sigma-Aldrich) as the positive control. The first 10 patient samples were tested with manual preparation of the cells. After establishing the ideal test parameters, 12 patient samples were tested by fully automated cell preparation, including a re-stain of PI after 24 h and 48 h to improve the detection of dead cells.

2.5. Data analysis

Based on the images and the fluorescence signals of the cells, viability analysis of CD138+ cells was performed for each microwell. The mean of all microwells of one condition was calculated, and the viability was normalized to the DMSO control. A normalized viability of 90 % was used as the cut-off for responders and non-responders. To determine the correlation with clinical data, R-ISS, lines of therapy (LOT), and previous treatment with the tested substance were compared to the *in vitro* response. Statistical analysis was performed using GraphPad Prism 9. All figures show the mean with the standard error of mean (SEM). The Mann-Whitney test was used when comparing two conditions, and the Kruskal-Wallis test with Dunn's multiple comparisons when there were more than two conditions. IC50 was calculated by non-linear regression using the standard slope.

3. Results

3.1. VivaCyte platform allows culturing of primary myeloma cells over 48 h

Ex vivo culturing of primary MM cells is challenging, and assessing the maximum culturing time is a relevant factor in *in vitro* drug testing platforms. Therefore, the human MM cell line U266 was incubated inside the instrument for 48 h to investigate their viability. The viability analysis was based on the PI signal of dead cells. With a viability of 87.0 % after 24 h and 60.1 % after 48 h, the culture conditions in the microwells were found to be suitable for MM cells (Fig. 1B).

Next, the viability of primary myeloma cells was tested by using MNC isolated from BM aspirates and CD138 fluorescence staining for MM detection. *In vitro* drug testing was performed on samples from 10 RRMM and 12 NDMM patients (detailed patients characteristics in Table 1). We observed a lower viability compared to U266, as expected, since primary myeloma cells are difficult to culture *ex vivo*.

The MM cells had a mean viability of 44.0 % (NDMM) and 39.2 % (RRMM) after 24 h (Fig. 1B). After 48 h, the viability further decreased to 20.5 % (NDMM) and 27.0 % (RRMM). For patient samples collected under therapy, the MM cell viability was only 14.3 % after 24 h and 10.0 % after 48 h. Due to poor viability or loss of CD138 expression, not all patient samples could undergo a response analysis at all time points. The number of MNC per microwell mainly ranged between 10 and 20.

Given the viability data, drug sensitivity analysis with the VivaCyte platform was applicable over a duration of 48 h for most patient samples.

3.2. In vitro response towards bortezomib, melphalan and dexamethasone was detectable in U266 and primary MM cells but not towards lenalidomide and mAb

In vitro drug responses were first investigated using U266 cells. Only bortezomib, dexamethasone and melphalan were used, as they all have a direct anti-MM effect. After 24 h, a significant increase of dead (PI+) cells was observed for bortezomib at concentrations of 50 nM and 200 nM, and for melphalan in all concentrations (Fig. 1C). This increase was even stronger after 48 h for melphalan, but not for bortezomib, as shown in Fig. 1D. In both cases, the rate of cell death increased in a dose-dependent manner. The IC50 after 24 h was 158.2 nM for bortezomib and 82.8 μM for melphalan; after 48 h, it was 161.5 nM for bortezomib and 9.7 μM for melphalan. In this setting, relatively high drug concentrations were necessary to observe drug responses. For dexamethasone, no significant difference in cell viability between the tested concentrations was seen (Fig. 1C-D). Since U266 is known to be resistant to dexamethasone, the drug testing successfully detected the expected response for all 3 drugs.

Next, we conducted a drug response screen with primary MM cells as shown in Fig. 1A. For bortezomib, we tested 10 patient samples, with 5 showing a significant increase in PI+ MM cells after 24 h (patient ID CP05, CP18, CP19, CP22, CP23), one only after 12 h (CP13), one after 16 h (CP20), one only after 48 h (CP16), and two being *in vitro* resistant to bortezomib (CP08, CP11; Fig. 2A). For the drug-sensitive patient samples, the median IC50 at 24 h was 12.5 nM. The *in vitro* sensitive patients (CP13, CP18, and CP19) showed at least a very good partial

Table 1
Demographic, baseline disease, and clinical characteristics of the patients. M=male, f=female, PI=proteasome inhibitor, Alk=alkylating agents, Dex=dexamethasone, IMiDs= immunomodulatory drugs, CD38=anti-CD38 mAb, Elo=elotuzumab.

Patient-ID	Age [years]	Gender	NDMM /RRMM	R-ISS	Myeloma type	Plasma cell infiltration in BM biopsy	Number of prior lines of therapy	Time since last therapy	Pre-exposed to substances class					
									PI	Alk	Dex	IMiDs	CD38	Elo
CP03	54	m	RRMM	II	IgA lambda	10 %	1	Under therapy	x	x	x			
CP04	46	f	NDMM	II	IgG kappa	80 %	-	-						
CP05	78	m	RRMM	III	IgG lambda	n.a.	1	2.5 months	x	x	x			
CP06	65	m	NDMM	II	IgG kappa	80 %	-	-						
CP07	82	f	RRMM	III	IgG kappa	70 %	2	13 months	x		x	X		
CP08	68	m	RRMM	I	IgA kappa	80 %	4	2 months	x	x	x	x		
CP09	68	m	NDMM	II	IgG lambda	90 %	-	-						
CP10	77	m	NDMM	n.a.	LC kappa	80 %	-	-						
CP11	78	m	RRMM	n.a.	IgG kappa	n.a.	4	Under therapy	x	x	x	x	x	x
CP13	64	m	NDMM	I	LC kappa	90 %	-	-						
CP16	67	f	NDMM	II	IgG kappa	95 %	-	-						
CP17	68	f	NDMM	II	LC kappa	80 %	-	-						
CP18	65	m	NDMM	II	IgG kappa	90 %	-	-						
CP19	58	m	NDMM	II	LC kappa	50 %	-	-						
CP20	69	m	RRMM	III	IgG lambda	<5 %	1	1 month	x		x	x	x	
CP21	69	m	NDMM	II	IgG kappa	40 %	-	-						
CP22	50	m	NDMM	I	IgG lambda	30 %	-	-						
CP23	65	m	RRMM	n.a.	IgG kappa	70 %	2	1.5 months	x	x	x	x	x	
CP24	77	m	RRMM	n.a.	IgG lambda	2 %	2	7 days	x		x	x	x	
CP25	54	m	RRMM	n.a.	IgG kappa	20 %	2	<4 weeks						
CP26	81	m	NDMM	I	IgG kappa	15 %	-	-	x	x	x	x	x	
CP27	69	f	RRMM	II	IgG lambda	80 %	5	3 weeks	x	x	x	x	x	

clinical response (VGPR) under a subsequently administered proteasome inhibitor (carfilzomib)-containing therapy. Patient CP16 reached only stable disease (SD) with an early relapse under a bortezomib combination. Patient CP05's bone marrow sample was drawn after an early relapse following bortezomib therapy with VGPR as the best clinical response. *In vitro* drug testing revealed that this patient might still have bortezomib-sensitive clones in the bone marrow. Patients CP20 and CP23 both received bortezomib as part of their previous line of therapy, showing a response and relapsing after bortezomib removal from combination therapy, supporting the *in vitro* results of remaining sensitivity towards bortezomib. Both resistant patients had advanced disease and were pretreated with bortezomib. In conclusion, for bortezomib, we could correctly identify all *in vivo* proteasome inhibitor responders and non-responders among the 8 patients with available clinical data.

Melphalan was tested in 7 patient samples, resulting in three responders (CP05, CP09, CP18) and four non-responders (CP11, CP13, CP16, CP19) after 24 h (Fig. 2B). The response could already be observed after 24 h with a median IC50 of 32.4 μM in responders. All the sensitive patients were naive to melphalan at the time point of bone marrow collection. CP09 showed melphalan sensitivity also *in vivo* since responding to a high-dose melphalan therapy with a partial response (PR) after quickly relapsing to all major drug classes before. Patient CP18 had a clinical long-term complete response (CR) after induction therapy and high-dose melphalan. Regarding the *in vitro* non-responders, none had previous melphalan exposure at the time of bone marrow puncture, although CP11 was heavily pretreated, including cyclophosphamide, another alkylating compound. CP16 achieved clinical only stable disease (SD) after receiving a cyclophosphamide-based therapy. Only CP13 underwent a high-dose melphalan therapy later, but since the patient already had a CR after the induction therapy, the benefit from melphalan is unknown. In conclusion, 4 out of 5 patients with clinical response data were correctly identified regarding their clinical response by the VivaCyte Beta platform.

Dexamethasone responses could be evaluated in 4 patients. All patient samples showed a significant *in vitro* response (Fig. 2C). The best measurable response was mostly reached after 24 h with a median IC50 of 25.2 μM. All patients were NDMM or in their first relapse and reached at least clinical VGPR under their dexamethasone-containing combination regimens.

In conclusion, in our setting, the VivaCyte Beta platform detected the effects of the cytotoxic drugs bortezomib, melphalan, and dexamethasone, which were mostly in concordance with the clinical responses of patients.

IMiDs and mAb are commonly used drug classes in MM therapy. We thus next examined the suitability of the VivaCyte platform to detect the anti-MM effect of these more complex acting substances.

Lenalidomide was tested in four patient samples for 12 h and in one for 24 h, identifying four as *in vitro* responders (CP04 after 24 h; CP08, CP25 and CP26 after 12 h) as shown in Fig. 2D. The median IC50 after 12 h was 170.5 μM for the responders. Decreases in the viability only reached significance for CP26 after 12 h and CP04 after 24 h. CP04 and CP26 were NDMM, CP04 achieved a clinical CR subsequently after lenalidomide-based triplet therapy. In contrast, *in vitro* sensitive patients CP08 and CP25 were clinically refractory to IMiDs, as well as the *in vitro* resistant patient CP27. Taken together, the results for IMiDs did identify 2 out of 4 patients with clinical data correctly and the IC50 was very high for all the patients.

The monoclonal antibodies daratumumab and elotuzumab were used in the *in vitro* platform for 4 patients. One patient sample (CP25) reached a significant response to daratumumab and to elotuzumab after 12 h (Fig. 2E-F), with an IC50 of 460.1 and 367.7 μg/ml respectively.

This patient was punctured at the timepoint of disease progression during daratumumab combination therapy, yet clinically not exposed to elotuzumab. Among the *in vitro* non-responders (CP06, CP26, CP27), 2 patients were NDMM (CP06 and CP26) with an expected initial response to the mAb. CP06 was treated with an elotuzumab therapy afterward and achieved a VGPR. The *in vitro* results for CP27 correlate to the previous therapies, since bone marrow was collected at disease progression under isatuximab, another anti-CD38 mAb.

Taken together, of patients with clinical response data, 50 % were assigned correctly by the platform for daratumumab, while elotuzumab was only administered to one patient *in vivo* in total. For elotuzumab, it is known that *in vivo* effect is only seen when combined with IMiDs [20], possibly explaining the failed response detection in our setting.

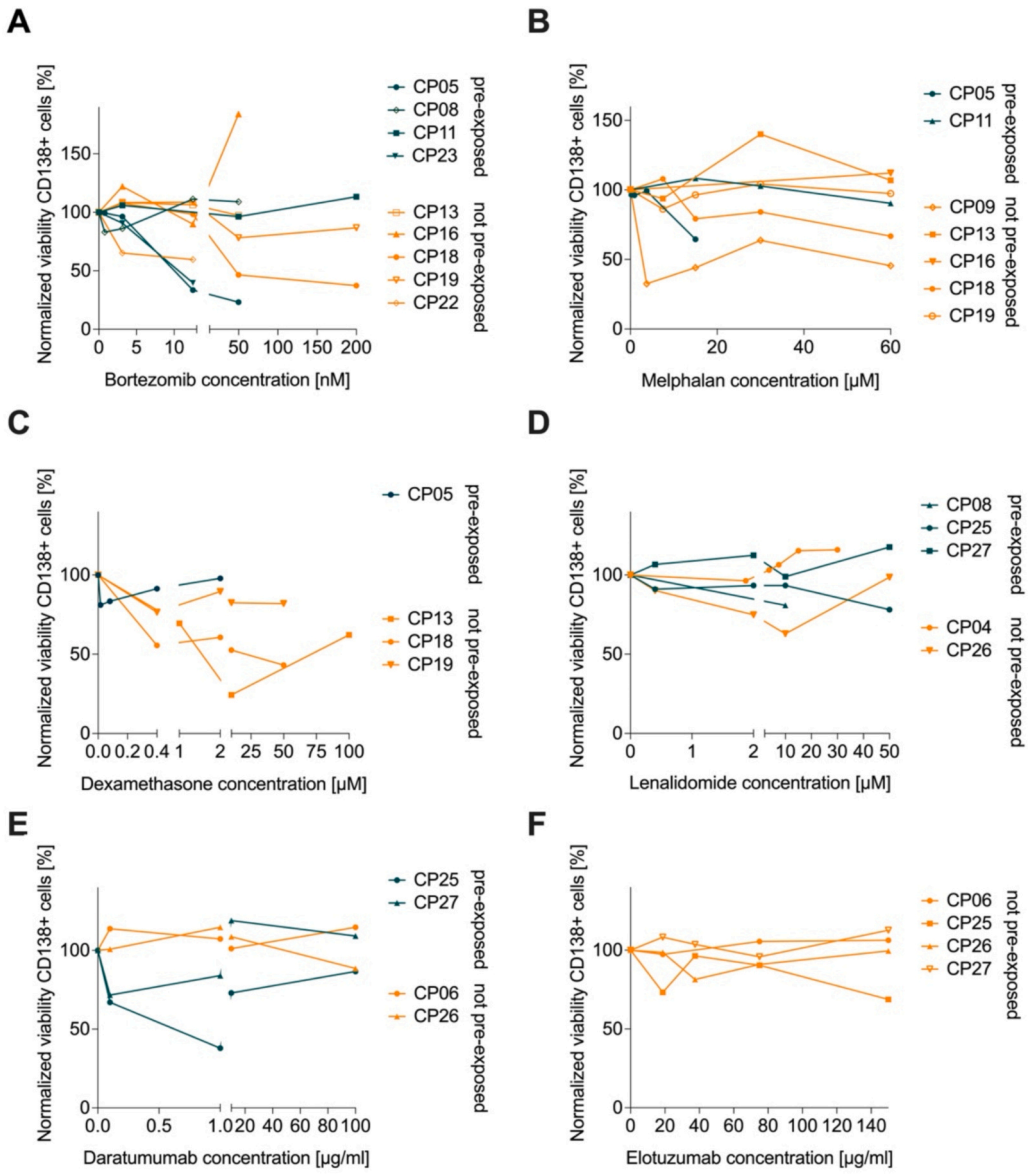

Fig. 2. *In vitro* drug responses of primary myeloma cells. Green graphs represent patients pre-exposed to the *in vitro* tested substance, orange graphs not pre-exposed patients. A-C. Dose-response curves of primary myeloma cells for (A) bortezomib, (B) melphalan and (C) dexamethasone with normalized viability after 24 h. D-F. Dose-response curves of primary myeloma cells for (D) lenalidomide, (E) daratumumab and (F) elotuzumab with normalized viability after 12 h.

For the more complex acting substances daratumumab, elotuzumab, and lenalidomide, the *in vitro* assessed responses did mostly not correlate with the clinical courses of the patients, and responses were only achieved with high drug concentrations. Therefore, it was not possible to perform this drug response screening assay for these three substances. This was most probably due to the short analyzable culturing time of just 12 h.

3.3. *Samples from heavily pretreated patients may have a higher rate of in vitro resistance*

To validate if the *in vitro* data show correlation to the clinical characteristics of the patients, the MM cell viability of each patient for each substance tested was compared to LOT, R-ISS, and pretreatment with the substance class. The direct correlation with the individual *in vivo* response to each drug was not possible since all patients received combinations of substances and the *in vivo* applied drugs did often not match with the drugs tested *in vitro*.

For the correlations, a score of response was defined using the lowest viability for each substance, independently from the time point or concentration. This allowed compensating for the variability of the cell viability obtained within the individual patients. A high *in vitro* response (MM viability <70 %) across all tested substances was seen in 68.4 % of cases among patients without any previous treatment, in 62.5 % of cases with a LOT of 1–2 and in 14.3 % of patients with a LOT > 2 (Fig. 3A). The highest proportion (42.9 %) of *in vitro* resistance (MM viability >90 %) was found in patients with > 2 LOT, while there was no case of resistance among treatment naïve patients. There was a trend to higher rates of *in vitro* resistance in patients with multiple relapses. This trend was also found investigating the individual substances. For bortezomib, melphalan, lenalidomide, daratumumab, and elotuzumab, the MM viability was lower after *in vitro* treatment in NDMM. The differences did not reach statistical significance, probably due to the low number of patients (Fig. 3B). There was also a trend for better responses to the substances bortezomib, lenalidomide, and dexamethasone when patients were not previously exposed to the drug class (Fig. 3C).

However, differences were not significant (bortezomib p=0.3095; IMiDs p=0.200). Regarding the correlation to the R-ISS and *in vitro* drug

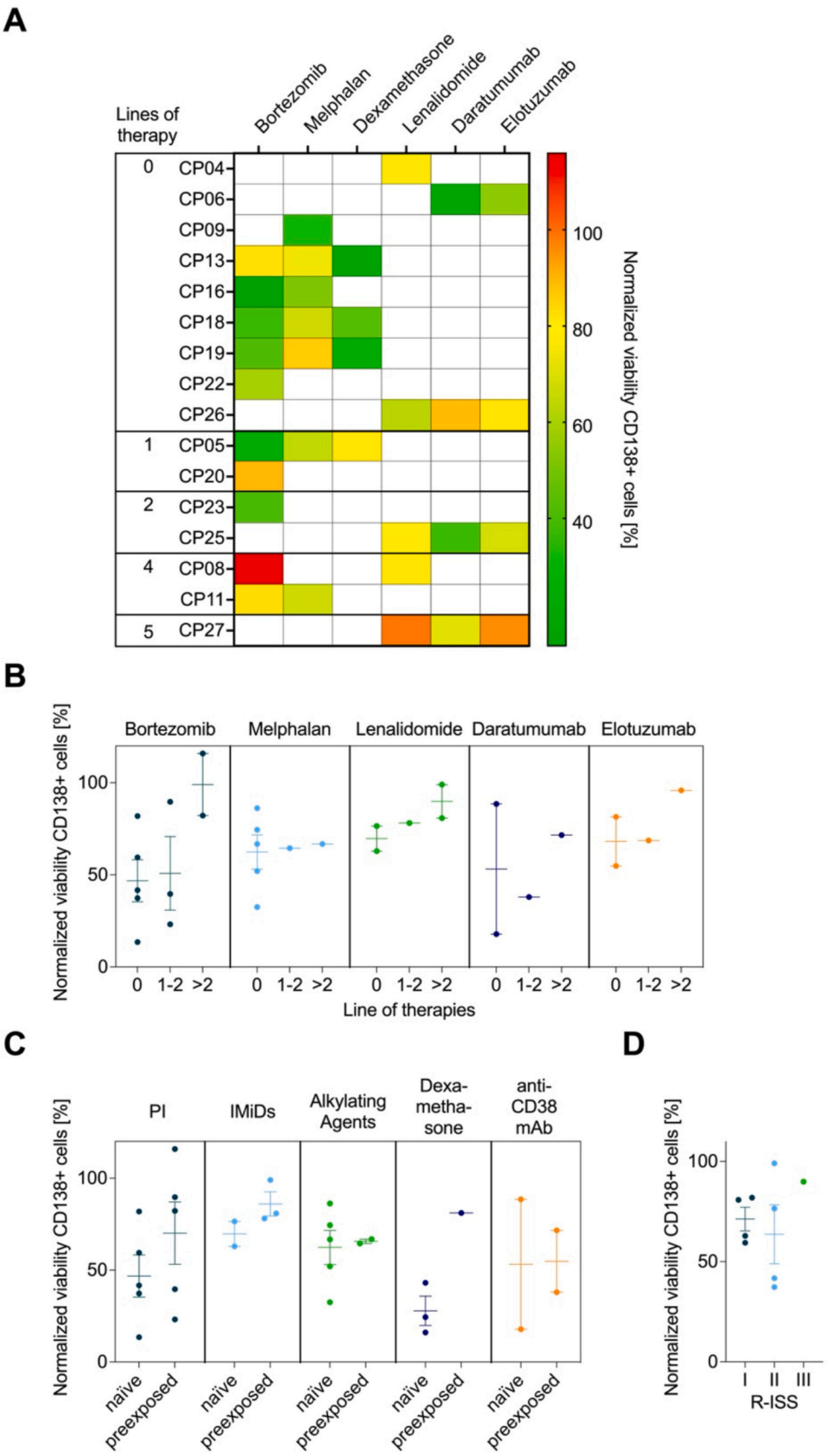

Fig. 3. Correlation of the best in vitro response with clinical data. A. Heatmap of the lowest normalized viability of each patient for the substances bortezomib, melphalan, dexamethasone, lenalidomide, daratumumab and elotuzumab. Data is sorted according to the lines of therapy (LOT). B-D. Correlation of lowest normalized viability of each patient with (B) number of prior lines of therapy for each tested substance, (C) with the pre-exposition towards each substance class and (D) with R-ISS. Shown is the mean + SEM. Kruskal-Wallis-Test with Dunn's multiple comparisons and Mann-Whitney-Test.

response, no correlation was found (Fig. 3D).

A correlation between the *in vitro* drug response and the number of therapy lines was evident for some patients. With the limited size of the testing cohort and the patient-to-patient variability, all differences appear as trends with no significant difference.

3.4. The VivaCyte platform identifies cellular cytotoxic effects of NK and T cells towards MM cells

Effector cells such as NK and T cells are relevant for the mode of action of both mAb daratumumab and elotuzumab, as well as for their resistance [12,15,16,21,22]. Additionally, novel therapies like CAR-T cells and T cell engagers require efficient T cell activity against MM cells. To explore if the VivaCyte platform can be used to determine the effector cell fitness using the existing microenvironment in the BM aspirates, we stained NK and T cells using antibodies against CD16, CD56, and CD3, and compared viability data between microwells with co-localization and cell-cell contact between myeloma cells and effector cells with those without an effector cell. Four patient samples (CP06, CP20, CP22, CP23) were tested for NK cell and two (CP08, CP13) for T cell effects. According to the known modes of action of the drugs, cells were treated with elotuzumab and daratumumab for NK cells [12,22,

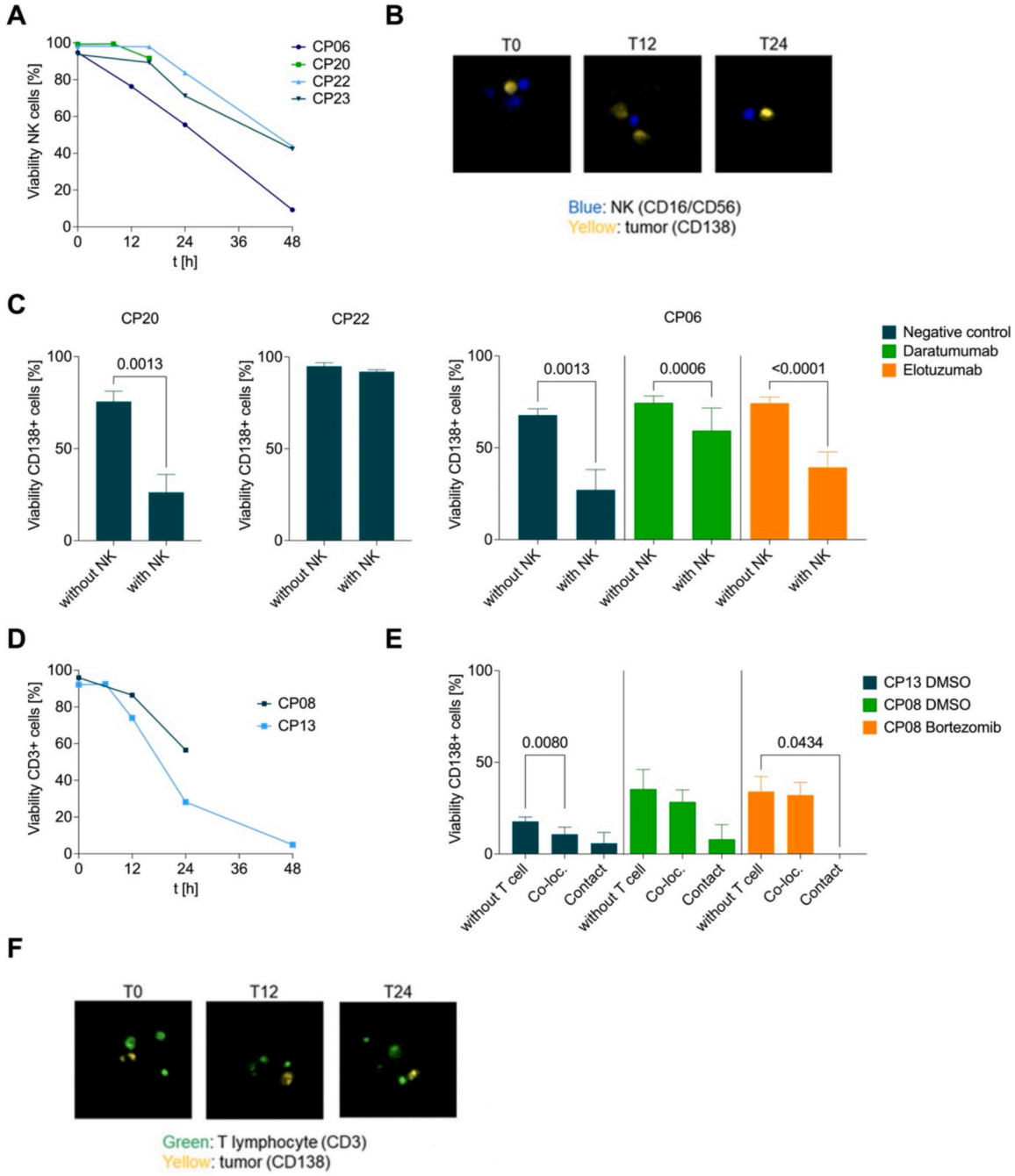

Fig. 4. Evaluation of MM directed NK and T cell activity. A. Viability of NK cells. B. Microscopic image of a microwell with NK cells (blue) and CD138+ cells (yellow) over 24 h. C. Viability of CD138+ cells after 12 h (CP06) or 16 h (CP20, CP22) in dependence of the co-localization of NK cells in negative control and treated with daratumumab or elotuzumab (CP06). Shown is the mean + SEM, Mann-Whitney-Test. D. Viability of T cells. E. Viability of CD138+ cells of two patients after 24 h in dependence of the co-localization with T cells treated with bortezomib or only in negative control. Shown is the mean + SEM, Kruskal-Wallis-Test with Dunn's multiple comparisons. F. Microscopic image of a microwell with T cells (green) and CD138+ cells (yellow) over 24 h.

23], and bortezomib for T cells [11].

The proportion of NK cells in the MNC was 8.3–12.5 % and 15–200 microwells per channel featured a co-localization of NK and tumor cells for all patients at every time point. This co-localization was demonstrated to be a cell-cell contact in almost every case. The maximum number of co-localizations of NK and tumor cells was observed at 0 h and 16 h. NK cell viability determined by PI signal ranged from 55.4 % to 83.7 % after 24 h (Fig. 4A) and was not influenced by any of the drugs used. An exemplary microwell with cell-cell contact between NK and MM cells is shown in Fig. 4B. In two patients (CP06, CP20), we found a significant increase of dead (PI+) MM cells after 12 h and 16 h when co-localized with NK cells compared to MM cells cultured in microwells without NK cells ($p<0.05$; Fig. 4C), suggesting NK cell efficacy in these patients. The viability of MM cells was reduced by the same amount in the negative controls as in the drug conditions, showing no increase in cytotoxicity by daratumumab or elotuzumab in CP06. CP06 achieved a VGPR under an elotuzumab-containing therapy, implying a good NK cell activity necessary for the elotuzumab effect *in vivo*. In CP22 and CP23's samples, we did not observe an increase of PI+ MM cells caused by NK cells, indicating possibly reduced NK cell anti-tumor activity *in vitro*. In the case of CP23, this could be attributed to prior therapy with daratumumab, as the mAb can also affect NK cells [21].

The proportion of T cells in the MNC was 25.3-29.5%, and 43–151 microwells featured a co-localization of T cells and tumor cells and 7–25 with cell-cell contact for each treatment condition.

The viability of CD3+ cells after 24 h was 56.5 % and 28.2 % for both patients (Fig. 4D) and was not decreased by the addition of bortezomib. Comparing myeloma viability in microwells with T cells and those without, the cell-cell contact of both cell types led to the highest rate of PI+ MM cells, as shown in Fig. 4E in both patients, possibly indicating good T cell efficacy. The T cell cytotoxicity against MM was not significantly increased by the addition of bortezomib in CP08, while other conditions showed significant differences (CP13 DMSO: 10.8 % vs. 17.8 %; $p=0.0080$ and CP08 bortezomib 0.0 % vs. 34.1 %; $p=0.0434$). An exemplary microwell with co-localization of multiple T cells with a myeloma cell is shown in Fig. 4F.

The VivaCyte platform thus demonstrated potential for exploring the NK and T cell cytotoxic activity against MM.

4. Discussion

In this study, the potential of the VivaCyte platform for *in vitro* drug response analysis with myeloma cells was assessed. The Cellply VivaCyte Beta platform demonstrated the capability to detect valid *in vitro* responses for bortezomib, dexamethasone, and melphalan, providing a fully automated high-throughput analysis approach suitable for larger drug screens. While *in vitro* results showed a high rate of correct identification of responders and non-responders, statistical significance was not reached due to the small dataset and high variation in *in vivo* administered therapeutics.

The quickly decreasing viability of myeloma cells resulted in a short culturing and assessment time. This limitation predominantly affected the drug response analysis for longer-acting drugs such as IMiDs and mAb. The work of Walker et al. demonstrated that an *in vitro* treatment duration of 48 h resulted in valid response data for all substance classes correlating with the clinical response data of patients [4]. Therefore, we decided to investigate the myeloma cells ex vivo without any further supplement. However, without additional support, myeloma cells could only be incubated for 48 h, with viability dropping to approximately 25 % after 48 h. Co-culturing MM cells with stromal cells could be a potential strategy to increase culture duration and needs further investigation. This approach showed successful expansion of incubation time by several days [24]. Supplementing IL-6 is another strategy that could successfully improve MM survival in the setting of *in vitro* drug testing platforms [4]. Additionally, for elotuzumab, a combination therapy evaluation may be necessary, as *in vivo* effects are only achieved by adding IMiDs [20]. Potentially, testing of combination therapy is feasible with the VivaCyte platform. Another approach for in vitro screening for drug responses in myeloma presented by Kropivsek et al. utilized cell morphology as a marker for cell death, known as pharmacoscopy. This strategy, detecting specific changes in cell morphology preceding cell death, showed significant correlation with clinical outcomes for all major drug classes in myeloma [5]. Changes in cell morphology may occur earlier than changes in the PI intensity used in this study, allowing for the observation of anti-proliferative effects even within shorter cultivation times.

The VivaCyte platform demonstrated the ability to detect T cells and NK cells, their cell-cell contacts with tumor cells, and their cytotoxic activity against MM cells. This capability enables screening for effector cell efficacy directly from unsorted bone marrow aspirates without the need for co-cultures with presorted cells. Analyzing effector cells in the drug response testing process is crucial, as they play a significant role in drug effects and may exhibit resistance mechanisms in advanced disease. In addition, this makes the VivaCyte platform an attractive tool to investigate novel CAR-T, CAR-NK or T cell receptor (TCR) therapies including both autologous and allogenic effector cells and T cell engagers which is subject on future applications of this platform.

Bettelli et al. demonstrated the assessment of ADCC of daratumumab using the Cellply platform [18]. Myeloma cells incubated with daratumumab exhibited lower viability when co-located with an NK cell in a microwell compared to without, highlighting the platform's suitability for analyzing response dependence of various cell types. Future studies should involve screening *in vitro* sensitivity towards drugs with additional commonly used drugs in a larger cohort to validate the predictive power of the platform and implement it as a predictive tool for clinical decision making.

In summary, in vitro drug sensitivity analysis automated by the VivaCyte platform emerges as a valuable tool for personalized medicine applications involving most myeloma drugs and may have the potential to determine effector cell effectivity for new cell therapies.

Contribution

J.Krüger conducted the experiments and analyzed the data. J.Krüger, A.B., L.R., M.B., A.N., U.K., O.B., J. Krönke, L.B. and I.W. B. designed the study. A. B., L. R. and M. B. performed the data generation by image analysis. J.Krüger and A.N. wrote the manuscript. All authors revised the manuscript and approved the final version.

CRediT authorship contribution statement

Igor Blau: Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Olga Blau:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Alice Bettelli:** Writing – review & editing, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Laura Rocchi:** Writing – review & editing, Software, Methodology, Data curation, Conceptualization. **Axel Nogai:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Lars Bullinger:** Conceptualization, Resources, Writing – review & editing. **Josefine Krüger:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Massimo Bocchi:** Writing – review & editing, Resources, Conceptualization. **Jan Krönke:** Writing – review & editing, Conceptualization. **Ulrich Keller:** Writing – review & editing, Conceptualization.

Declaration of Competing Interest

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founder of Cellply S.r.l. The remaining authors declare no financial interest/relationships to this article.

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