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CONCISE COMMUNICATION

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Evaluation of Sézary cell marker expression and cell death behaviour upon in vitro treatment by flow cytometry in Sézary syndrome patients

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Abstract

The diagnosis of Sézary syndrome (SS) relies on the identification of blood Sézary cells (SC) by different markers via flow cytometry. Treatment of SS is challenging since its pathogenesis is characterized by cell death resistance rather than hyperproliferation. In this study, we establish an integrated approach that considers both the expression of SC markers and sensitivity to cell death both spontaneously and upon in vitro treatment. Peripheral blood mononuclear cells were isolated from 20 SS patients and analysed for the SC markers CD7 and CD26 loss as well as CD158k and PD1 gain. The cells were then treated with different established and experimental therapies in vitro

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and cell death was measured. Spontaneous and therapeutically induced cell death were measured and correlated to cellular marker profiles. In the marker-positive cells, spontaneous cell death sensitivity was reduced. Different treatments in vitro managed to specifically induce cell death in the putative CTCL cell populations. Interestingly, a repeated analysis after 3 months of treatment revealed the CTCL cell death sensitivity to be restored by therapy. We propose this novel integrated approach comprising the evaluation of SC marker expression and analysis of cell death sensitivity upon treatment that can also enable a better therapy stratification.

KEYWORDS
cell death resistance, cutaneous T cell lymphoma, dimethyl fumarate, Sézary syndrome, therapy stratification

1 | BACKGROUND

Mycosis fungoides (MF) and Sézary syndrome (SS) are the most prevalent primary cutaneous T cell lymphoma (CTCL) entities.[1,2] In 2007, a revised staging and classification system was introduced with the TNMB classification that considered blood involvement separately.[3] Sézary cells (SC) can be either identified by morphological examination of blood smears or by flow cytometry and BIOMED-II analysis of T cell receptor (TCR) clonality, the defining feature of the malignant SC.[4,5] Several markers for the identification of SC by flow cytometry have been identified and characterized, such as loss of CD7 and CD26, as well as gain of killer cell immunoglobulin-like receptor 3DL2 (KIR3DL2/CD158k) and programmed death protein 1 (PD1/CD279).[6–10] However, it is yet to be clarified which of these markers characterizes the malignant cell population best so that a standardized marker panel for the identification of SC can be established.[11]

To date, SS poses a therapeutic challenge since the patients rapidly develop resistance to conventional chemotherapeutic approaches and show high relapse rates even under highly efficacious therapy. A reason for this seems to be a distinct resistance of the malignant T cells towards cell death.[12–17] Therefore, restoration of cell death sensitivity and subsequent cell death induction is a central therapeutic approach in the treatment of CTCL. In vitro evaluation of collected SC for cell death sensitivity upon treatment with a selected drug would enable therapy stratification prior to treatment and thus accelerate targeted depletion of the tumour load.

2 | QUESTIONS ADDRESSED

In this study, peripheral blood samples collected from SS patients were analysed for the SC marker expression and their cell death behaviour both spontaneously and upon in vitro treatment with CTCL therapeutics. Thereby we propose a novel integrated flow cytometric approach that enables an individualized therapeutic stratification by an in vitro approach even before starting therapy.

3 | EXPERIMENTAL DESIGN

Twenty SS patients (CTCL stage IV) diagnosed according to World Health Organization (WHO)—European Organization for Research and Treatment of Cancer (EORTC) classification of CTCL and criteria of the International Society for Cutaneous Lymphomas (ISCL) were included in this study.[1] Fourteen patients diagnosed with Mycosis fungoides (MF) and twelve psoriasis patients were included as controls. Written informed consent was collected from all patients and the data were analysed anonymously. The study was conducted according to ethical guidelines at our institution and the Declaration of Helsinki and was approved by the Ethics Committee II of the University of Heidelberg (reference number 2018-653N-MA) and by the ethical committees of the participating centers. A characterization of the patient cohort is provided in Table 1.

Whole blood samples were collected from the patients and peripheral blood mononuclear cells (PBMCs) were isolated as described before.[18] The blood samples from the CTCL patients were taken under systemic CTCL therapy, apart from six CTCL patients on therapy break at the timepoint of the sample collection (Table 1). The PBMCs were harvested within 24 h after blood sampling and seeded at a concentration of 2.5×10^9 cells/L in RPMI medium in FACS tubes and treated with dimethyl fumarate (DMF) (50 µmol/L), bexarotene (3 µmol/L), and mitomycin c (20 nmol/L) (Sigma Aldrich). The concentration of the therapeutics was determined by pre-experiments for cell death that did not reach saturation. DMF has been successfully evaluated in CTCL in a clinical phase II study with an overall response rate of 30.4% in the skin compartment.[19] Bexarotene is a 'rexinoid' that was specifically developed and approved for the treatment of CTCL in advanced stages.[20,21] Mitomycin c as alkylating agent acts as a potent DNA crosslinker[22] and is currently used for the treatment of different tumour entities.[23–25] We used it in our study, as it induces apoptosis, but not other forms of cell death.

The cells were stained for the established Sézary cell markers CD7, CD26, CD158k and PD1.[6–10] The applied antibodies are provided in Table S1. Clonality of the TCR Vβ chains was assessed with the Beta Mark TCR Vβ Repertoire Kit (IM3497, Immunotech SAS, Marseille)

TABLE 1 Clinical data of the Sézary syndrome patients.

Patient	Gender, age (years)	TNM stage	Leukocytes/nL	T lymphocytes	$CD4^+$ cells/μL	SC/μL	CD4/CD8 ratio	% BSA	Treatment
1	Male, 77	T4NxM0B2	95.7	99.60%	78445	69145	71.9	80	ECP, Alem
2[a]	Female, 58	T4NxM0B2	6.7	77.70%	na	1084	20.3	20	ECP, Alem
3	Male, 71	T4N2M0B2	8.6	30.80%	na	1997	34.3	60	ECP, IFN
4[a]	Male, 79	T4NxM0B2	10.2	25.20%	na	756	15.9	80	ECP
5	Male, 72	T4N0M0B2	24.4	98.90%	na	15235	98.9	20	Brent
6[a]	Female, 69	T4N3M0B2	10.1	91.60%	na	4235	76.3	20	Bex
7	Female, 78	T4NxM0B2	12.4	95.00%	4613	860	11.6	>80	ECP[a], Ali
8[b]	Male, 61	T4N3M0B2	8.68	94.00%	2739	1585	19.4	>80	ECP, Moga, Ali
9[a]	Female, 68	T4NxM0B0	8.7	89.00%	2050	355	5	>80	ECP, Ali
10[a]	Male, 61	T4NxM0B0	4.55	81.00%	442	116	2	>80	ECP, Ali
11	Male, 83	T4cNxM0B2	16.23	95.10%	10974	5172	40.2	33	ECP, MTX
12	Female, 65	T4NxM0B2	8.59	93.60%	3252	3125	43.8	63	ECP, IFN
13	Male, 73	T4NxM0B2	6.17	92.30%	3223	2966	35.7	0	Moga
14[b]	Female, 57	T4N0M0B2	6.4	96.40%	2951	2481	28.4	mSWAT 47	ECP[a]
15	Female, 71	T4NXM0B2	7.79	94.40%	3651	844	26.7	mSWAT 73	ECP
16[b]	Male, 71	T4NXM0B2	6.65	95.20%	2058	1931	82.1	mSWAT 58	ECP, IFN, Bex
17[b]	Male, 76	T4N0M0B2	12.71	93.40%	4449	384	21.6	mSWAT 53	ECP
18	Female, 83	T4NXM0B2	10.95	89.10%	3110	2550	60.8	70	MTX
19[b]	Male, 70	T4N2M0B2	8.9	95.50%	7524	7308	81.8	mSWAT 73	DMF
20[b]	Female, 70	T4N1M0B0	7.4	84.30%	1129	937	7.4	mSWAT 85	DMF
Norm			4.2–10.2	60%–83%	528–1495	<1000	1–2.8		

Abbreviations: Alem, alemtuzumab; Bex, bexarotene; Brent, brentuximab; BSA, body surface area; DMF, dimethylfumarate; ECP, extracorporeal photophoresis; IFN, interferone; Moga, mogamulizumab; MTX, methotrexate; na, not available; SC, sézary cells. BSA, mSWAT and laboratory results are provided from the time point of the peripheral blood sample collection.

[a]No PD1 staining available.

[b]Repeated spontaneous cell death measurement after 3 months of treatment.

according to the manufacturer's instructions.[26] Nomenclature for clones and corresponding Vβ segments was applied as postulated by Wei et al.[27] Cell death was measured by forward-to-side scatter (FSC/SSC) profile after 0, 24 and 48 h.[28] Untreated cells were prepared and measured as controls. Representative histograms and FACS plots from the FSC/SSC and 7-AAD/Annexin V stainings are provided in Figure S2–S4. The statistical analyses were calculated with GraphPad Prism (GraphPad Software, San Diego, California). The differences were considered significant at $p < 0.05$, and the level of significance is indicated by asterisks (**** ≤ 0.0001; *** ≤ 0.001; ** ≤ 0.01 and * ≤ 0.05).

4 | RESULTS

Blood samples of SS patients were analysed for their expression of Sézary immunophenotype markers. A typical Sézary immunophenotype was defined by typical expression of the one assessed marker independently from the expression of the other markers. Marker positivity was measured according to the recommendation of the International Society for Cutaneous Lymphomas (ISCL) and the European Organization for Research and Treatment of Cancer (EORTC) Cutaneous Lymphoma Working Group.[8,29] Here, the B2 stage in SS patients is defined by expanded $CD4^+$ T cells with abnormal immunophenotype including loss of CD7 or CD26 (≥40% $CD4^+$ $CD7^-$ or ≥30% $CD4^+$ $CD26^-$). CD158k positivity was defined as ≥30% $CD4^+$ $CD158k^+$, PD1 positivity was defined as ≥25% in the $CD4^+$ cell population, while Vβ clonality was defined as ≥75%.

A per definition measurable CD7 loss was detected in 65% of the samples, CD26 loss in 95%, and CD158k gain in 70% of the patient samples. Strikingly, 100% of the SC patient samples expressed PD1 (Figure 1A). The mean quantitative expression of CD7 was 53.4%, of CD26 80.7%, of CD158k 52.9% and of PD1 92.6% (Figure 1B). The SC of 60% of patients expressed all four markers ($CD7^-$ $CD26^-$ $CD158k^+$ $PD1^+$), additional 20% patients expressed three markers, and 20% of patients expressed only two markers. No SS patient expressed only one marker or no marker (Figure 1C). Ninety percent of SS patients showed detectable Vβ clonality upon flow cytometry. Consequently, the expression of the distinct clonal Vβ chains was analysed. We found that 35% of the SS patients expressed Vβ 2, 30% expressed Vβ 5.1 and

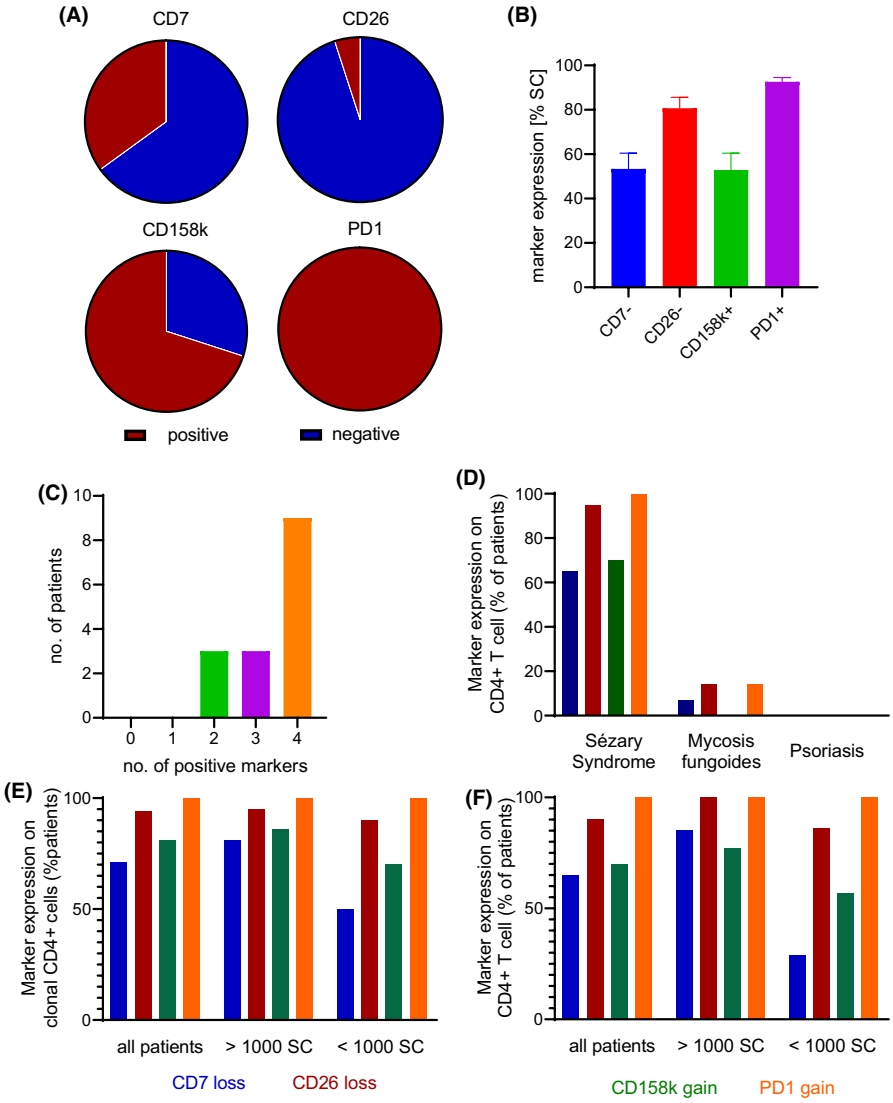

FIGURE 1 Evaluation of Sézary marker expression in primary Sézary syndrome patient samples. (A) Differential positivity of the SS patients for the Sézary markers CD7 loss, CD26 loss, CD158k gain, PD1 gain. A typical Sézary immunophenotype was defined as $CD4^+CD7^-$, $CD4^+CD26^-$, $CD4^+CD158k^+$ or $CD4^+PD1^+$ compared to cells with an atypical immunophenotype ($CD4^+CD7^+$, $CD4^+CD26^+$, $CD4^+CD158k^-$, $CD4^+PD1^-$). (B) Mean quantitative expression of CD7 loss, CD26 loss, CD158k gain and PD1 gain in the $CD4^+$ cell population of SS patients. (C) Number of SC markers ($CD7^-$, $CD26^-$, $CD158k^+$, $PD1^+$) expressed by the $CD4^+$ T cells of the SS patients. $N = 20$ SS patients for the CD7, CD26, and CD158k stainings, $n = 15$ SS patients for the PD1 stainings. (D) Marker expression (CD7 loss, CD26 loss, CD158k gain, PD1 gain) on $CD4^+$ T cells in Sézary syndrome in percent of patients ($n = 20$) compared to MF ($n = 14$) and psoriasis patients ($n = 12$) as controls. PD1 expression was measured for $n = 12$ MF and $n = 10$ psoriasis patients. (E) Comparison of the marker expression (CD7 loss, CD26 loss, CD158k gain, PD1 gain) of Sézary syndrome patients with high (> 1000 SC per μL) and low tumour load (<1000 SC per μL). Percentage of the Sézary marker-positive clonal $CD4^+$ T cells from the complete $CD4^+$ T cells in Sézary syndrome patients with >1000 compared to <1000 Sézary cells per μL (for CD7, CD26 and CD158k analysis: All patients $n = 18$; > 1000 SC per μL $n = 12$; < 1000 SC per μL $n = 6$; for PD1 analysis: All patients $n = 14$; > 1000 SC per μL $n = 10$; < 1000 SC per μL $n = 4$). (F) Percentage of the Sézary marker-positive $CD4^+$ T cells from the complete $CD4^+$ T cells in Sézary syndrome patients with >1000 compared to <1000 Sézary cells per μL (for CD7, CD26 and CD158k analysis: All patients $n = 20$; > 1000 SC per μL $n = 13$; < 1000 SC per μL $n = 7$; for PD1 analysis: All patients $n = 15$; > 1000 SC per μL $n = 11$; < 1000 SC per μL $n = 4$).

Vβ 8, while 10% expressed not further characterized Vβ chains (Figure S1A), so there is a predisposition for certain Vβ chains to clonally expand in CTCL.

Then, the marker expression of the $CD4^+$ T cells from SS patients was compared to MF patients and psoriasis patients as controls. Psoriasis is a benign chronic inflammatory condition. Whereas we found the typical SC marker expression in the SS samples as described above, in MF, a CD7 loss was detected in only 7% of the samples, and CD26 loss and PD1 gain in 14% respectively. No MF patient had a CD158k gain in the $CD4^+$ T cell population. In psoriasis, no $CD4^+CD7^-$, $CD4^+CD26^-$, $CD4^+CD158k^+$ and $CD4^+PD1^+$ cells were detected (Figure 1D).

Consequently, the SS cohort was separated in patients with high (>1000 SC/μL) and low tumour burden (<1000 SC/μL) and the SC marker expression was correlated to clonality. We found the percentage of marker-positive patient samples very similar in the $CD4^+$ and Vβ−clonal populations in patients with high tumour load, which confirms the stability of the markers for diagnostic purposes. In patients with low tumour burden, we found higher variability in the percentage of marker-positive cells for CD7, CD26 and CD158k between both analyses (Figure 1E,F). Here, the clonality as a reference seems necessary, as in the Vβ clonal population, the marker positivity is reliably high compared to the complete $CD4^+$ population, most probably due to a high percentage of non-malignant bystander T cells. Intriguingly, PD1 is expressed on all Vβ positive and $CD4^+$ Sézary cells independently of the tumour load.

Then, cell death behaviour was analysed. First, the rate of spontaneous cell death in the $CD4^+$ T cells was measured and categorized according to marker positivity. In the $CD7^-$ cells spontaneous cell death was significantly reduced compared to the $CD7^+$ cells. For the $CD26^-$ cells a trend towards reduced cell death was detected, although not reaching statistical significance ($p = 0.09$), whereas CD158k expression did not influence cell death sensitivity (Figure 2A). Thereafter, the cells were treated in vitro with three different drugs for 24 h and the difference in cell death compared to untreated controls was calculated. We found DMF to significantly induce specific cell death in the $CD7^-$ and $CD158k^+$ and thus SS-typical cell populations compared to the respective marker-negative cell populations (Figure 2B).

Subsequently, the ratio of cell death in marker-positive compared to marker-negative cell populations was calculated for each marker (e.g. $CD7^-/CD7^+$, $CD26^-/CD26^+$, $CD158k^+/CD158k^-$) after in vitro treatment for 24 h. Treatment with DMF induced cell death in the marker-positive cell populations compared to the untreated controls, while bexarotene induced cell death in the $CD7^-$ and $CD158k^+$ cells, but not in the $CD26^-$ cells. For mitomycin c, increased cell death induction could not be shown in the marker-positive cell populations (Figure 2C) supporting the literature and clinical experience that chemotherapy is hardly effective in CTCL.[30] The raw data are provided in Figure S1B–D. Here, multiple statistically significant results were identified between the marker-positive cell population and the controls upon treatment with DMF, bexarotene and mitomycin c.

Additionally, for six SS patients the cell death behaviour in the $PD1^+$ cell population was assessed (Figure 2D). Bexarotene and mitomycin c induced significantly increased cell death in the marker-positive, $PD1^+$ cell populations. DMF treatment induced comparably high cell death rates in both the $PD1^+$ and the $PD1^-$ cell population in the FSC/SSC profile. Additional 7AAD/Annexin V stainings revealed that late apoptosis was significantly increased in the $PD1^+$ cell population (Figure S1E).

Subsequently, the spontaneous cell death rates were measured after 3 months of treatment in six patients. Interestingly, no differences in the spontaneous cell death rates between marker-positive and marker-negative cells could be detected anymore (Figure 2E).[21] Thereby we could show that on a short-term level of 3 months, that CTCL therapy at least attenuates the characteristic cell death resistance of SC. A graphical summary of the results is provided in Figure 3.

5 | CONCLUSIONS AND PERSPECTIVES

To date there is no objective universal definition of SC in flow cytometry. In addition to loss of CD7 and CD26, different markers like CD158k[31–33] and PD1[10,34–36] are used to define the malignant T cell population the best possible, but the differential quality of these markers for diagnostic purposes is not yet fully evaluated.[37] Vergnolle et al. found in a multivariate flow cytometry analysis that CD158k and PD1 expression might define heterogeneous subtypes of SS, stressing the importance of additional novel markers apart from CD7 and CD26 loss for the correct and unique identification of SC.[38] In our patient cohort, especially in patients with low SC count (<1000 SC/μL), we confirmed that a combination of different surface markers as well as clonality analysis seems to be necessary for a clear diagnosis in accordance with the literature.[4,39,40]

Interestingly, we could detect PD1 expression in 100% of the cells with a typical Sézary immunophenotype and its expression was independent from the SC count. No psoriasis patient expressed PD1, while in MF patients a PD1 gain was detected in 14% of the samples, which corresponded to CD26 loss. Luherne et al. found significantly increased PD1 expression in skin biopsies from SS patients compared to erythrodermic patients with benign inflammatory erythroderma.[41] However, Roelens et al. identified benign $CD4^+$ $PD1^+$ T cells in the peripheral blood of SS patients and a varying PD1 expression depending on the treatment.[42]

The heterogeneous treatment and the inter-individual heterogeneity of the SS patients might influence the SC marker profile which explains the differential marker expression and why only a marker combination and no singular marker seems to be suited for diagnostics.[43,44] Nevertheless, several questions are still to be answered and the diagnostic profile in SS blood diagnostics is still to be optimized. Therefore, the EORTC's Cutaneous Lymphoma Working Group is collaborating with the EuroFlow consortium to develop a standardized, accurate and highly sensitive flow cytometry protocol for the identification of SC in patients with CTCL and exploration of tumour heterogeneity with already promising first results.[37,44,45] With this study we aim to contribute to a standardized approach for the measurement of blood involvement in CTCL for diagnosis and staging, as well as the evaluation of treatment response.

Although it is scientifically acknowledged that resistance to cell death outweighs hyperproliferation in CTCL, the SS patients' benign and malignant T cell populations have not yet been directly compared intra-individually. In our study, we were able to show that the rate of spontaneous cell death is decreased in all marker-positive, supposedly malignant, T cell populations compared to the respective marker-negative bystander T cell population.

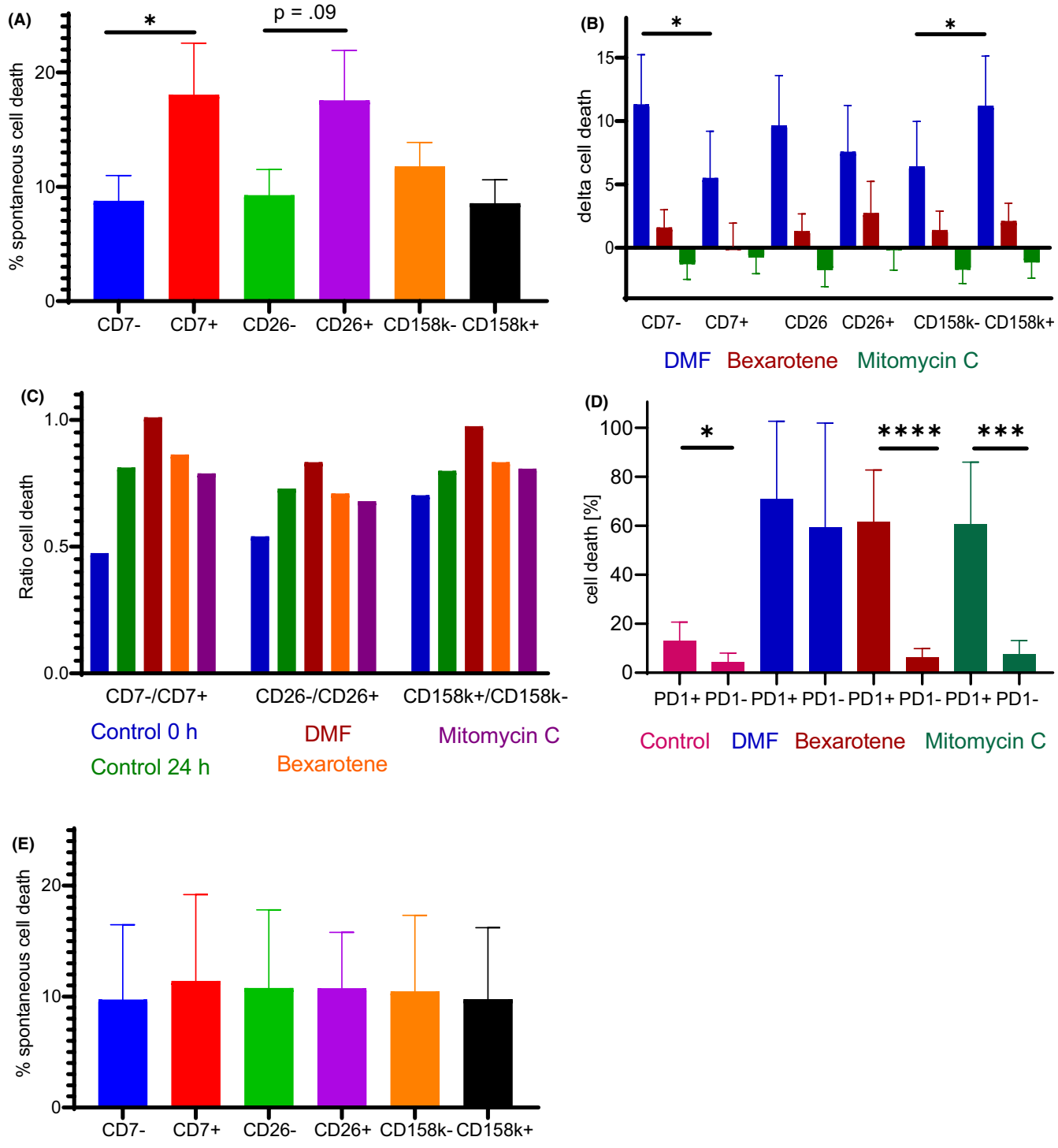

FIGURE 2 Analysis of cell death behaviour in the cells with Sézary marker expression ($CD7^-$, $CD26^-$, $CD158k^+$) compared to cells without Sézary marker expression ($CD7^+$, $CD26^+$, $CD158k^-$) derived from SS patients. (A) Spontaneous, unstimulated cell death (in percent) in the cells with Sézary marker expression intra-individually compared to cells without Sézary marker expression ($CD7^+$, $CD26^+$, $CD158k^-$) ($n = 20$). (B) Specific cell death (referred to unstimulated controls) after 24 h of stimulation with DMF, bexarotene, or mitomycin c in cells with Sézary marker expression compared to cells without Sézary marker expression ($n = 20$). (C) Ratio of the mean cell death rate in the marker-positive cells compared to marker-negative cells after stimulation with DMF, bexarotene or mitomycin ($n = 20$). Unstimulated controls were prepared at the time points 0 and 24 h. (D) Therapeutically induced cell death in the cells with PD1 expression compared to the $PD1^-$ cells collected from $n = 6$ SS patients. (E) Spontaneous cell death (in percent) in $CD4^+$ T cells with Sézary marker expression ($CD7^-$, $CD26^-$, $CD158k^+$) compared to cells without Sézary marker expression ($CD7^+$, $CD26^+$, $CD158k^-$) after 3 months of stage-adapted CTCL therapy ($n = 6$). All results are depicted as mean values plus standard deviation. **** ≤ 0.0001; *** ≤ 0.001; ** ≤ 0.01 and * ≤ 0.05. Bexa, bexarotene; DMF, dimethyl fumarate; Mito, mitomycin C.

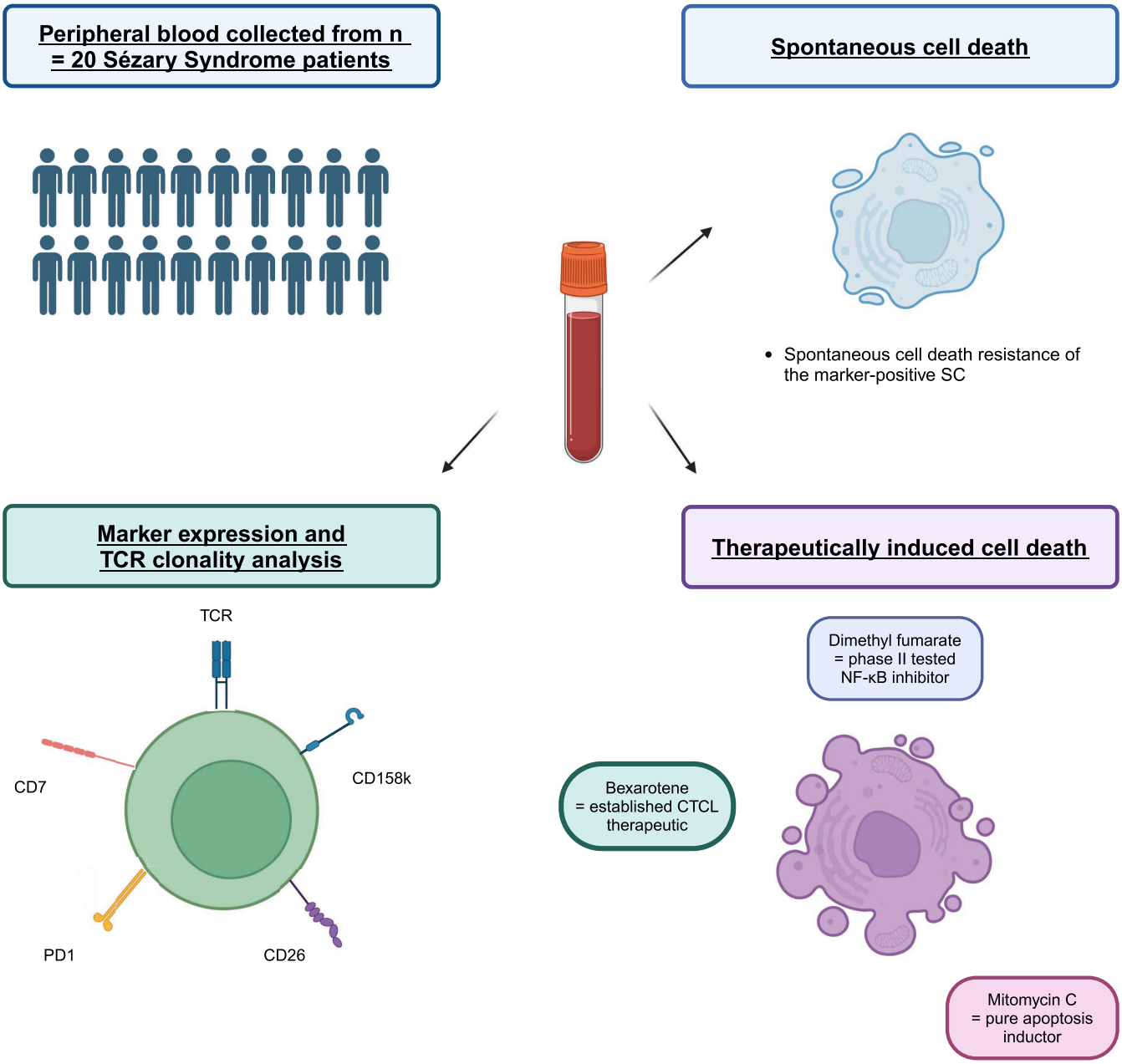

FIGURE 3 Graphical summary of the study. Peripheral blood was collected from human patients and the marker expression was analysed as well as spontaneous and therapeutically induced cell death. This figure was created with BioRender.

Treatment with DMF was able to induce increased specific cell death in the $CD4^+CD7^-$, $CD4^+CD26^-$, and $CD4^+CD158k^+$ T cells compared to treatment with bexarotene and mitomycin c after 24 h of stimulation. For the $CD4^+PD1^+$ and the $CD4^+PD1^-$ T cells comparably high cell death rates were detected. DMF is a promising novel CTCL therapeutic that showed good clinical responses in the skin compartment and excellent tolerability in CTCL patients in a clinical phase II study.[19] The effect was markedly stronger in the $CD4^+CD7^-$, $CD4^+CD26^-$, and $CD4^+CD158k^+$ cell population pointing to an enhanced sensitivity of the malignant cells towards the treatment and to a specific pro-apoptotic effect of DMF on the malignant population. This can be explained by the mechanism of action: DMF blocks the transcription factor NF-κB that is aberrantly activated in SC, but not in benign T cells and thus has a very specific CTCL-directed effect.[18,46] For the $CD4^+PD1^+$ no significant difference could be detected compared to the marker-negative cell population in the FSC/SSC profiles. However, 7AAD/Annexin V stainings revealed a significant increase in the late apoptotic cell population in the marker-positive $PD1^+$ cells. We attribute this observation to the pharmacokinetics of DMF since DMF induces cell death very rapidly.[18,47]

The negative values in specific cell death for bexarotene and mitomycin c describe a decrease in cell death rate compared to spontaneous cell death and might be explained by different effects: first, the short duration of the stimulation (24 h), which might be too short for bexarotene and mitomycin c to reach their full activity, since bexarotene modulates transcriptional activity and mitomycin c is a prodrug that needs to be activated firstly. Second, the cells are taken from their microenvironment that also influences their cell death resistance.[48,49] Furthermore, we could show by our analysis after 3 months of treatment that the relative resistance to cell death of the marker-positive cell populations was not detectable anymore. This finding illustrates that different systemic CTCL therapies can restore sensitivity of the malignant T cells towards cell death.

Our novel integrated approach enables us to differentially investigate cell death in putative malignant and benign T cell populations which generates novel information on the biology of CTCL. Additionally, CTCL treatments can thereby be evaluated in vitro to individually identify the treatment with high efficacy in the malignant population while leaving the benign bystanders unaffected. Therefore, it is a first step towards therapeutic stratification of patients prior to therapy.

AUTHOR CONTRIBUTIONS

Conception and design: J.P.N. *Acquisition of data*: S.M., M.R., J.D.A., C.A., C.v.G., E.G., C.D.K., R.C.K.M., M.S., R.S., U.W., M.W., T.A., S.G., S.S., J.P.N. *Analysis and interpretation of the data*: S.M., M.R., J.D.A., T.A., J.P.N. *Drafting the article*: S.M., M.R., J.P.N. *Revising the article critically for important intellectual content*: S.M., M.R., J.D.A., C.A., C.v.G., E.G., C.D.K., R.C.K.M., M.S., R.S., U.W., M.W., T.A., S.G., S.S., J.P.N. *Final approval of the version to be published*: S.M., M.R., J.D.A., C.A., C.v.G., E.G., C.D.K., R.C.K.M., M.S., R.S., U.W., M.W., T.A., S.G., S.S., J.P.N.

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CONFLICT OF INTEREST STATEMENT

J.P.N. received travel and congress participation funding from TEVA and Novartis, as well as consulting fees from TEVA, Almirall, Biogen, Novartis, and Kyowa Hakko Kirin, Innate Pharma, Takeda, and Actelion. S.M. received honoraria from Kyowa Kirin. M.W. received funding for congress participation and consulting fees by Kyowa Kirin, Takeda, Recordati Rare Diseases and Stemline Therapeutics. C.D.K. received funding for congress participation and consulting fees by Actelion, BMS, Innate Pharma, Kyowa Kirin, MSD, Novartis, Recordati, Roche, Stemline, Takeda, TEVA and Therakos. R.K.C.M. received travel and congress participation funding from Kyowa Hakko Kirin and Recordati rare diseases, as well as consulting fees from TEVA, Kyowa Hakko Kirin, Recordati rare diseases, and Takeda. E.G. received honoraria and/or grant support from Mallinckrodt, Helsinn, Takeda, Novartis, and Kiowa unrelated to this work. C.A. is active as consultant/advisory for Takeda, Kyowa, Helsinn, Recordati, and 4SC. U.W. has performed consultancies for Takeda, Therakos, Kyowa Kirin, Recordati Rare Diseases, Stemline, Mundipharma, Helsinn and Galderma, and lectured at educational events sponsored by MSD, Takeda, Galderma, Kyowa Kirin, Stemline and Recordati Rare Diseases.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

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

Figure S1. (a) Distribution of TCR-Vβ chain clonality in $n = 20$ Sézary syndrome patients (in % of patients). (b–d) Raw data of the analysis of cell death behaviour in the cells with Sézary marker expression (CD7− (b), CD26− (c), CD158k+ (d)) compared to cells without Sézary marker expression (CD7+ (b), CD26+ (c), CD158k− (d)) derived from SS patients upon treatment with DMF, bexarotene and mitomycin C Ratios from these raw data are summarized and shown in Figure 2C (e). Therapeutically induced cell death (late apoptosis) measured by 7AAD/Annexin V in the CD4+ T cells with PD1 expression compared to the PD1− cells collected from $n = 6$ SS patients.

Figure S2. Histogram examples for the definition of CD7+/−, CD26+/−, CD158k+/−, and PD1+/− from a representative SS, MF and psoriasis patient.

Figure S3. Representative FSC/SSC plots for the CD26− compared to the CD26+ cell population after 24 h of in vitro DMF treatment.

Figure S4. Representative 7-AAD/Annexin V stainings for the CD26− compared to the CD26+ cell population after 24 h of in vitro DMF treatment.

Table S1. Antibodies used for the FACS stainings. † Antibodies used for the analysis of the PD1+ cell fraction. ‡ Antibodies used for the cell death analysis.

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