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The impact of SAMHD1 expression and mutation status in mantle cell lymphoma: An analysis of the MCL Younger and Elderly trial

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Abstract

The sterile alpha motif and histidine-aspartic domain-containing protein 1 (SAMHD1) has been demonstrated to predict the response to high-dose cytarabine consolidation treatment in acute myeloid leukemia patients. Here, we evaluated SAMHD1 as potential biomarker for the response to high-dose cytarabine in mantle cell lymphoma (MCL) patients. We quantified SAMHD1 protein expression and determined the mutation status in patients of the MCL Younger and Elderly trials (n = 189), who had received high-dose cytarabine- or fludarabine-based polychemotherapy. Additionally, we quantified SAMHD1 expression in B cell lymphoma cell lines and exposed them to cytarabine, fludarabine, and clinically relevant combinations. Across both trials investigated, SAMHD1 mutations had a frequency of 7.1% (n = 13) and did not

Abbreviations: AML, acute myeloid leukemia; ASCT, autologous stem cell transplantation; BL, Burkitt lymphoma; CI, confidence interval; CLL, chronic lymphocytic leukemia; CR, complete remission; DAB, diaminobenzidine; DLBCL, diffuse large B cell lymphoma; DMSO, dimethyl sulfoxide; dNTP, deoxynucleoside triphosphate; ECOG, Eastern Cooperative Oncology Group; EDTA, ethylenediaminetetraacetic acid; EMCLN, European Mantle Cell Lymphoma Network; FBS, fetal bovine serum; FFS, failure-free survival; HR, hazard ratio; LDH, lactate dehydrogenase; MCL, mantle cell lymphoma; MFI, median fluorescence intensity; MIPI, Mantle Cell Lymphoma International Prognostic Index; OR, odds ratio; OS, overall survival; PBS, phosphate-buffered saline; R-CHOP, rituximab, cyclophosphamide, vincristine, doxorubicine, prednisolone; R-DHAP, rituximab, dexamethasone, high-dose cytarabine, cisplatin; R-FC, rituximab, fludarabine, cyclophosphamide; RPMI, Roswell Park Memorial Institute-1640; SAMHD1, sterile alpha motif and histidine-aspartic domain-containing protein 1; TMA, tissue microarray.

significantly affect the failure-free survival (FFS, $P = .47$). In patients treated with high-dose cytarabine- or fludarabine-containing regimes, SAMHD1 expression was not significantly associated with FFS or complete remission rate. SAMHD1 expression in B cell lymphoma cell lines, however, inversely correlated with their in vitro response to cytarabine as single agent ($R = .65$, $P = .0065$). This correlation could be reversed by combining cytarabine with other chemotherapeutics, such as oxaliplatin and vincristine, similar to the treatment regime of the MCL Younger trial. We conclude that this might explain why we did not observe a significant association between SAMHD1 protein expression and the outcome of MCL patients upon cytarabine-based treatment.

KEYWORDS

B cell lymphoma, cytarabine, mantle cell lymphoma, resistance, SAMHD1

1 | INTRODUCTION

Mantle cell lymphoma (MCL) is an aggressive B cell lymphoma which is characterized by early dissemination and unfavorable prognosis.¹ The introduction of rituximab and intensified treatment approaches, such as autologous stem cell transplantation (ASCT), significantly improved the prognosis of MCL.^{2,3} During recent years, the European Mantle Cell Lymphoma Network (EMCLN) conducted two important studies, which contributed to establish the current treatment standard for MCL patients: First, the MCL Younger trial demonstrated that the addition of high-dose cytarabine improves failure-free survival (FFS) of patients aged 65 years or younger.⁴ Second, the MCL Elderly trial showed that the combination of rituximab, fludarabine and cyclophosphamide (R-FC) is inferior to rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) in terms of overall survival (OS) in patients older than 65 years who are not eligible for high-dose therapy.⁵ In addition, the MCL Elderly trial demonstrated that maintenance therapy with rituximab significantly prolonged OS.⁵

Despite improved treatment standards, MCL shows a continuous pattern of recurrence and is therefore still regarded as an incurable disease.⁶ One third of the patients relapses within 5 years after treatment initiation of which almost 40% were reported to suffer from a chemotherapy-refractory disease.^{7,8} Even though several prognostic factors including the Mantle Cell Lymphoma International Prognostic Index (MIPI) are well-established,⁹ the biological factors underlying early or late relapse kinetics are not completely understood.

Recently, the sterile alpha motif and histidine-aspartic domain-containing protein 1 (SAMHD1) was reported as a biomarker predicting the response to high-dose cytarabine consolidation therapy in acute myeloid leukemia (AML) patients.¹⁰⁻¹² SAMHD1 is a triphosphohydrolase that cleaves deoxynucleoside triphosphate (dNTPs) into deoxyribonucleosides and inorganic triphosphate. High expression of SAMHD1 leads to enhanced hydrolysis of the active metabolite of cytarabine, and thus counteracts its cytotoxic effects.¹² Apart from cytarabine, structurally similar antiproliferative agents,

What's new?

While mantle cell lymphoma (MCL) treatment has improved, relapse and unfavorable outcome remain problematic. Here, to better predict therapeutic response in MCL, the authors assessed the biomarker potential of the nucleotide metabolism enzyme SAMHD1. Analyses of patients in the MCL Younger and Elderly trial show that neither SAMHD1 protein expression nor mutation status are associated with failure-free survival or complete remission rate upon cytarabine-based treatment in MCL patients. Drug perturbation assays in B cell lymphoma cell lines suggest that high SAMHD1 expression mediates cytarabine resistance as monotherapy but not when combined with other chemotherapeutics, similar to MCL treatment in vivo.

such as fludarabine, nelarabine or decitabine are also presumed substrates of SAMHD1,^{13,14} potentially limiting their efficacy in the treatment of hematologic malignancies. However, SAMHD1 is also described as tumor suppressor,¹⁵ and reduced expression, as a consequence of promotor methylation, was reported to be associated with Sézary syndrome¹⁶ and lung adenocarcinoma.¹⁷ Likewise, SAMHD1 mutations result in a reduced mRNA and protein expression¹⁸ and were identified as potential drivers of oncogenesis in chronic lymphocytic leukemia (CLL).¹⁹

In our study, we hypothesized that high SAMHD1 expression negatively impacts the response to high-dose cytarabine- or fludarabine-based induction treatment in MCL patients. To address this clinically relevant question, we quantified the SAMHD1 protein expression and determined the mutation status in 189 patients of the MCL Younger and Elderly trial. We analyzed the impact of SAMHD1 expression and mutation on the outcome of MCL patients treated with fludarabine- or cytarabine-containing treatment. We further treated B cell lymphoma cell lines with cytarabine, fludarabine, and clinically relevant

combinations, and investigated the effect of SAMHD1 expression on the response to these drugs and drug combinations.

2 | METHODS

2.1 | Procession of fresh lymph node samples

Immediately after the excision, the lymph node was cut in small pieces and put into RPMI-1640 supplemented with 10% FBS, penicillin and streptomycin at a final concentration of 100 U/mL and 100 µg/mL and L-glutamine at a final concentration of 2 mM. After filtering by a 40 µm strainer, cells were washed once with phosphate-buffered saline (PBS) and put into RPMI medium supplemented with 20% fetal bovine serum (FBS) and 10% dimethyl sulfoxide (DMSO, Serva), and then cryopreserved in liquid nitrogen until further analysis. All growth medium components and PBS were obtained from Gibco.

2.2 | Intracellular flow cytometry staining of SAMHD1

Primary lymph node-derived cells were thawed and washed once with FBS supplemented with 1% FBS and 0.5% Ethylenediaminetetraacetic acid (EDTA). Then, the cells were stained with fixable viability dye e506 (Thermo Fisher Scientific), and anti-CD3-APC/Cy7 and anti-CD19-PE/Cy7 antibodies (all Biolegend). After washing, cells were fixed and permeabilized by 60 minutes incubation with ice cold 90% Methanol. The cells were stained with 1 µg rabbit anti-SAMHD1 antibody (12586-1-AP, Proteintech) or 1 µg corresponding rabbit isotype control (Thermo Fisher Scientific) followed by donkey anti-rabbit-FITC secondary antibody (Biolegend). For cell lines, surface staining was not performed. Cells were then analyzed with an LSR II (BD Biosciences). The polyclonal rabbit-derived anti-SAMHD1 antibody (12586-1-AP, Proteintech) was validated in lymphoma cell lines using a monoclonal anti-SAMHD1 antibody (MA5-25298, Invitrogen, clone OTI1F6). See Supplementary Figure 1 for validation experiments.

2.3 | Staining of SAMHD1 on tissue microarrays

Immunohistochemical staining of SAMHD1 was performed on 3 µm thick sections of tissue microarrays (TMAs) consisting of duplicate 0.6 mm cores using a semiautomated tissue stainer (LEICA) with conventional DAB staining. After heat-induced epitope retrieval at pH 9.0 the rabbit polyclonal SAMHD1 antibody (1:200; Proteintech) was incubated for 30 minutes, followed by washing and detection according to the manufacturer's protocol. SAMHD1 was found to be strongly positive in T cells and was scored by two experienced hematopathologists as 0/negative, no staining; 1+, positive reaction in the majority of B blasts, but weaker as in T cells; 2+, moderate to strong staining in B blasts equivalent to or stronger as in T cells.

2.4 | Automated image analysis of TMAs

Slides stained for SAMHD1 were scanned using a Hamamatsu NanoZoomer Digital Pathology system (Hamamatsu). Then, the staining intensity of SAMHD1 was quantified using the open source software QuPath (v0.1.2) according to the recommended workflow.²⁰ After detection of all cells per TMA core, the measurement data were exported and further analyzed using R. The intracellular signal of diaminobenzidine staining of all detected events was visualized in a histogram and revealed two separated peaks for the majority of cores, indicating two populations which express SAMHD1 at different levels. Comparing SAMHD1-stained slides with CD3-stained slides revealed that the SAMHD1^{High} population corresponds to T cells. This assumption was confirmed by intracellular flow cytometry staining of SAMHD1.

2.5 | Assessing the SAMHD1 mutation status

Sequencing for SAMHD1 mutations was done by the EuroClonality-NGS DNA capture (EuroClonality-NDC) panel, developed by the EuroClonality-NGS working group.²¹ Briefly, libraries were constructed from 200 ng of genomic DNA using the KAPA Hyperplus Kit with Library Amplification (KAPA Biosystems) and SeqCap Adapter Kit A & B (Roche Sequencing Solutions). Hybridization of the libraries was performed using the custom designed SeqCap EZ Choice Probes. Enriched libraries underwent sequencing on an Illumina NextSeq 500 (Illumina) using a paired end 75 bp approach.

2.6 | Statistics

The evaluation of potential prognostic and predictive effects of SAMHD1 expression on clinical outcome (complete remission, CR, rate, failure-free survival, FFS) in subsets of the MCL Younger and Elderly trials was of exploratory nature. CR rate was defined as percentage of patients with CR at end of induction immunochemotherapy among those with a staging result. FFS was calculated from start of induction treatment to the first event, defined as stable disease during induction, progressive disease, or death from any cause. Patients without observed FFS event were censored at the latest contact date at which lymphoma progression was excluded. For two-group comparisons of numerical and binary variables, Mann-Whitney *U* tests and exact Fisher's tests were performed, respectively. Survival distributions were displayed using Kaplan-Meier estimates and compared between groups with the log-rank test. Bivariable associations with Ki-67 index were explored using Spearman's correlation coefficient. Logistic and Cox regression were used to explore the prognostic value of SAMHD1 expression on CR rate and FFS, respectively. To explore potential predictive effects, the interaction term of treatment group and SAMHD1 expression was included besides treatment group and SAMHD1 expression in the respective regression model. MIPI⁹ score was included for adjustment in the analyses of the predictive value of SAMHD1 expression on FFS. *P* values <.05 were formally called

statistically significant; however, differences with larger *P*-values could be considered suggestive for potential effects case-by-case with respect to the exploratory nature of our study.

2.7 | Cell lines

Human B cell lymphoma cell lines were kindly provided by Martina Seiffert [German Cancer Research Center Heidelberg: OCI-Ly3 (RRID: CVCL_8800), OCI-Ly19 (RRID: CVCL_1878), SU-DHL-4 (RRID: CVCL_0539), SU-DHL-5 (RRID: CVCL_1735)], and by Thorsten Zenz [University of Zürich; MAVER-1 (RRID: CVCL_1831), BL-60 (RRID: CVCL_7034), Z-138 (RRID: CVCL_B077), Raji (RRID: CVCL_0511), Mino (RRID: CVCL_1872), UPN1 (RRID: CVCL_A795), Granta-519 (RRID: CVCL_1818), TMD8 (RRID: CVCL_A442), HLY-1 (RRID: CVCL_H207), DoHH2 (RRID: CVCL_1179), U-937 (RRID: CVCL_0007), JVM-2 (RRID: CVCL_1319)]. Z-138 were cultured in Iscove's Modified Dulbecco's Medium (IMDM), supplemented with 10% horse serum; TMD8 and OCI-Ly19 were cultured in alpha Minimal Essential Medium supplemented with 10% FBS; Granta-519 were cultured in Dulbecco's modified Eagle medium, supplemented with 10% FBS; OCI-Ly3 were cultured in Roswell Park Memorial Institute-1640 medium (RPMI, Gibco), supplemented with 20% FBS; SU-DHL-4, SU-DHL-5, MAVER-1, BL-60, Raji, Mino, UPN1, HLY-1, DoHH2, U-937 and JVM-2 were cultured in RPMI-1640, supplemented with 10% FBS. All media were additionally supplemented with 2 mM L-glutamine, 100 IU/mL penicillin and 100 mg/mL streptomycin. Cells were incubated at 37°C and 5% CO₂. All growth medium components were obtained from Gibco. The cell culture supernatants of all cell lines were regularly tested for mycoplasma by PCR. All experiments were performed with mycoplasma-free cells. Cell lines were authenticated within the last 3 years using Multiplex Cell Authentication by Multiplexion (Heidelberg, Germany) as described recently.²² The SNP profiles matched known profiles or were unique.

2.8 | Western blot and protein quantification

Cells were washed twice with cold PBS and lysed with radio-immunoprecipitation assay buffer (Sigma-Aldrich) supplemented with phosphatase inhibitor cocktail and protease inhibitor cocktail (both from Roche). The protein concentration was determined by a detergent compatible protein assay (Biorad) according to the manufacturer's instructions. The protein lysates were subjected to SDS-PAGE separation and subsequently transferred onto a polyvinylidene difluoride membrane (Thermo Fisher Scientific). The membranes were incubated with 5% milk in Tris-buffered saline for 1 hr and probed overnight at 4°C with rabbit anti-SAMHD1 (12586-1-AP, Proteintech) or mouse anti-β-actin (66009-1-Ig, Proteintech). After washing with TBS supplemented with 0.05% Tween (Sigma) three times, the membranes were incubated with goat anti-rabbit (SA00001-2, Proteintech) or goat anti-mouse secondary antibodies (SA00001-1, Proteintech) for 1 hr and detected using the Amersham Imager 600 (Healthcare

Life Sciences). The bands were quantified using the open source software ImageJ.^{23,24} SAMHD1 expression of each cell line was calculated by the mean intensity of three independent western blots. The polyclonal rabbit-derived anti-SAMHD1 antibody (12586-1-AP, Proteintech) was validated in lymphoma cell lines using a monoclonal anti-SAMHD1 antibody (MA5-25298, Invitrogen, clone OTI1F6). See Supplementary Figure 1 for validation experiments.

2.9 | In vitro drug perturbation assays

Cells were seeded at 5000 cells/well in a 384 well plate in the presence of a single drug or drug combinations for 48 hours. Cells were treated with cytarabine (32 nM, 160 nM, 800 nM, 4000 nM) or fludarabine (80 nM, 400 nM, 2000 nM, 10 000 nM), combining with or without cyclophosphamide (40 nM, 200 nM, 1000 nM, 5000 nM), dexamethasone (40 nM, 200 nM, 1000 nM, 5000 nM), doxorubicin (8 nM, 40 nM, 200 nM, 1000 nM), oxaliplatin (40 nM, 200 nM, 1000 nM, 5000 nM) and vincristine (40 nM, 200 nM, 1000 nM, 5000 nM) separately. Except for cyclophosphamide, drugs were purchased from Selleck Chemicals. As cyclophosphamide itself is a prodrug and not active in vitro, the active metabolite 4-hydroxyperoxy-cyclophosphamide (Niomech) was used. In contrast to the MCL Younger trial, we used oxaliplatin instead of cisplatin, because both compounds are inactivated by DMSO,²⁵ and oxaliplatin but not cisplatin is soluble in water. Final DMSO concentrations did not exceed 0.5%. Cell viability was determined by using the ATP-based CellTiter Glo assay (Promega).

3 | RESULTS

3.1 | Patient characteristics of the subsets of the MCL Younger and MCL Elderly study cohort

To evaluate the impact of SAMHD1 expression on the response to cytarabine- or fludarabine-based treatment, we assembled a meaningful cohort of MCL patient samples. Based on the availability of TMA slides, we selected 106 out of 404 MCL patients treated in the MCL Younger trial and 83 out of 251 MCL patients, who were treated in the MCL Elderly trial by German study centers. Supplementary Tables 1 and 2 compare all relevant patient characteristics between the German trial cohorts and the subgroups selected for this analysis, including age, sex, stage, Eastern Cooperative Oncology Group (ECOG) performance status, lactate dehydrogenase (LDH), leukocyte count, MIPI and Ki-67. Our subgroup selected from the MCL Younger trial harbored patients who were younger (*P* = .001), had a lower MIPI score (*P* = .0004) and a lower leukocyte count (*P* = .016) compared to those excluded. The FFS benefit for patients treated with R-CHOP/R-DHAP (rituximab, dexamethasone, cisplatin, high-dose cytarabine) compared to the patients who received R-CHOP only induction treatment—as reported for the entire cohort⁴—was maintained in our subgroup (Supplementary Figure 2). Our subgroup selected from the MCL Elderly trial was also comparable with the cohort of patients excluded,

except for the number of stage IV patients, who were enriched in our subgroup ($P = .048$, Supplementary Table 2). The OS benefit for R-CHOP compared to R-FC was maintained in our study subgroup, but did not reach statistical significance due to reduced statistical power ($P = .1$). Taken together, we considered the selected study subsets from the MCL Younger and Elderly trial to be representative for the entire study populations.

3.2 | SAMHD1 quantification in lymph node biopsies

We quantified SAMHD1 expression by immunohistochemistry and automated image analysis using TMA with two separate cores per

patient. In parallel, we stained for CD3, to identify T cell-rich areas. Figure 1A,B shows a representative staining for SAMHD1 and CD3 of the same core. The bimodal distribution of the SAMHD1 intensity suggested two individual populations with markedly different expression levels of SAMHD1 (Figure 1C). To understand this bimodal distribution, we systematically compared the cores stained for SAMHD1 with those stained for CD3, and we observed that SAMHD1 staining intensity was significantly enhanced in T cell-rich areas (Figure 1A,B). To validate this finding, we isolated single cell solutions from fresh lymphoma lymph node biopsies and stained them for CD3, CD19, viability and SAMHD1. We observed that T cells had significantly higher expression levels of SAMHD1 than B cells ($n = 8$, $P = .01$). These results indicate that lymph node-derived B and T cells can be distinguished based on SAMHD1 expression levels. For the purpose of our

FIGURE 1 Automated image analysis-based quantification of SAMHD1 expression in the cohorts of the MCL Younger and MCL Elderly trial. A and B, Immunohistochemical staining of SAMHD1 (A) or CD3 (B) in one representative mantle cell lymphoma TMA core. Red arrows highlight CD3⁺, T cell-rich areas. C, The histogram shows all cells of panel A by staining (DAB) intensity of SAMHD1. The two peaks represent the two different cell populations, as indicated. D, Primary B cell lymphoma lymph node-derived cells were stained for viability, CD3, CD19 and SAMHD1. Plot shows the SAMHD1 levels of B cells and T cells of the same lymph node ($n = 8$). The P value was calculated by the two-sided Wilcoxon test. E, A total of 378 TMA cores (two per patient) were stained for SAMHD1. The positivity was then evaluated by automated image analysis as described in panels A to C (see Section 2 for details) and by two independent pathologists with a ranking of 0, 1 or 2. Each dot represents the mean intensity of two cores (automated analysis, y axis) or the mean score of both pathologists (x axis). For each discrete level (x axis), additional box plots are depicted. DAB, diaminobenzidine [Color figure can be viewed at wileyonlinelibrary.com]

study, we quantified SAMHD1 expression only within the B cell subset (Figure 1C). We further validated our results obtained by automated image analysis by two experienced hematopathologists who rated the SAMHD1 expression in tumor cells of each stained TMA core manually between zero and two, resulting in five different discrete scores per patient (0, 0.5, 1, 1.5, 2). Figure 1E illustrates that the continuous value obtained by the automated analysis correlated well with the pathologist's estimate.

3.3 | SAMHD1 expression correlates with Ki-67 expression in MCL

First, we correlated the SAMHD1 expression levels across both study trials with the most important risk factors established for MCL, including the MIPI score, its components (age, ECOG, LDH, white blood count), and the proliferation marker Ki-67 ($n = 150$, Supplementary Table 3). We found that neither the MIPI score nor its components were significantly associated with SAMHD1 expression levels. Only the proliferation marker Ki-67 moderately correlated the SAMHD1 expression levels in MCL biopsies (Spearman's $\rho = 0.26$, $P = .0015$).

3.4 | Impact of SAMHD1 on the outcome of MCL Younger and Elderly trial patients

We further investigated whether SAMHD1 expression affects the CR rate of patients treated in the MCL Younger and Elderly trial. By

fitting a logistic regression model, we found that high SAMHD1 expression levels were not associated with lower CR rates in patients treated with high-dose cytarabine in the MCL Younger trial (odds ratio, OR, 2.9 for SAMHD1 increased by 1, $P = .16$, Table 1). Similarly, there was no impact of SAMHD1 expression on the CR rate of patients treated with R-CHOP only induction in the MCL Younger trial (OR 1.9, $P = .62$), and patients treated with R-FC (OR 1.8, $P = .58$) or R-CHOP (OR 1.1, $P = .92$) in the MCL Elderly trial (Table 1). For both trials, the estimated OR for the impact of SAMHD1 expression on achieving a CR with R-CHOP/R-DHAP or R-FC, respectively, were greater than 1, which contrasts to our initial hypothesis that high SAMHD1 levels are associated with a reduced CR rate.

Next, we analyzed the prognostic and predictive value of SAMHD1 expression with regard to FFS. In the MCL Younger trial, the FFS was not significantly associated with SAMHD1 expression in both treatment arms with a hazard ratio (HR) of 0.80 for R-CHOP/R-DHAP (SAMHD1 increased by 1, $P = .73$) and a HR of 1.8 for R-CHOP/R-CHOP, (SAMHD1 increased by 1, $P = .36$, Table 2). Figure 2 shows the Kaplan-Meier survival curves for four different prespecified value ranges of SAMHD1 expression levels (≤ 0.5 , > 0.5 and ≤ 0.67 , > 0.67 and ≤ 1 , > 1) for the R-CHOP only arm (Figure 2A) and the R-CHOP/R-DHAP arm (Figure 2B). Consistent with the results from Cox regression, we did not observe any differences between these groups in both treatment arms. In the Cox regression models, the HR of R-CHOP/R-DHAP vs R-CHOP were below one ($HR < 1$). For increasing SAMHD1 expression levels we observed decreasing HR for the comparison of R-CHOP/R-DHAP vs R-CHOP (Supplementary Table 4); however, this interaction did not reach statistical significance (interaction $P = .38$).

TABLE 1 Logistic regression model of complete remission

Trial	Treatment group	CR/patients	Regression coefficient	OR (95% CI)	P value
MCL Younger	R-CHOP/R-DHAP + ASCT	20/58	1.0733	2.9 (0.66-13)	.16
MCL Younger	R-CHOP/R-CHOP + ASCT	7/31	0.6491	1.9 (0.15-25)	.62
MCL Elderly	R-FC	14/37	0.5659	1.8 (0.24-13)	.58
MCL Elderly	R-CHOP	10/42	0.1242	1.1 (0.11-12)	.92

Note: Regression coefficients, odds ratios (OR) with 95% confidence intervals (CI) and P values for logistic regression of probability to achieve a complete remission (CR) in treatment subgroups of the two trials. The odds ratio indicates the comparison to an increase by 1 in SAMHD1 expression. The interaction P -values for differential effects between the two treatment groups by SAMHD1-expression were $P = .78$ in both trials.

Abbreviations: ASCT, autologous stem cell transplantation; R-CHOP, rituximab, cyclophosphamide, doxorubicine, vincristine, prednisolone; R-DHAP, rituximab, dexamethasone, cisplatin, high-dose cytarabine; R-FC, rituximab, fludarabine, cyclophosphamide.

TABLE 2 Cox regression model of failure-free survival

Trial	Treatment group	Events/patients	Regression coefficient	HR (95% CI)	P value
MCL Younger	R-CHOP/R-DHAP + ASCT	20/60	-0.22672	0.80 (0.23-2.8)	.73
MCL Younger	R-CHOP/R-CHOP + ASCT	23/32	0.55830	1.7 (0.54-5.7)	.36
MCL Elderly	R-FC	27/39	0.26702	1.3 (0.43-3.9)	.64
MCL Elderly	R-CHOP	27/42	-1.33942	0.26 (0.07-0.95)	.0416

Note: Regression coefficients, hazard ratios (HR) with 95% confidence intervals and P values for Cox regression of failure-free survival in treatment subgroups of the two trials. The HR indicates the comparison to an increase by 1 in SAMHD1 expression. The interaction P values for differential effects between the two treatment groups by SAMHD1 expression were $P = .38$ in MCL Younger, and $P = .014$ in MCL Elderly.

Abbreviations: ASCT, autologous stem cell transplantation; R-CHOP, rituximab, cyclophosphamide, doxorubicine, vincristine, prednisolone; R-DHAP, rituximab, dexamethasone, cisplatin, high-dose cytarabine; R-FC, rituximab, fludarabine, cyclophosphamide.

FIGURE 2 The impact of SAMHD1 expression on the failure-free survival of patients treated in the MCL Younger trial. Kaplan-Meier curves show the probability of failure-free survival within the subsetted MCL Younger cohort in the R-CHOP/R-CHOP arm (A) or R-CHOP/R-DHAP arm (B) according to SAMHD1 expression levels (four groups). The curves are colored by different SAMHD1 expression levels: blue (1): ≤ 0.5 ; green (2): > 0.5 and ≤ 0.67 ; gray (3): > 0.67 and ≤ 1 ; and black (4): > 1 . ASCT, autologous stem cell transplantation; R-CHOP, rituximab, cyclophosphamide, doxorubicine, vincristine, prednisolone; R-DHAP, rituximab, dexamethasone, cisplatin, high-dose cytarabine; R-FC, rituximab, fludarabine, cyclophosphamide

FIGURE 3 The impact of SAMHD1 expression on the failure-free survival of patients treated in the MCL Elderly trial. Kaplan-Meier curves show the probability of failure-free survival within the subsetted MCL Elderly cohort in the R-CHOP arm (A) or R-FC arm (B) according to SAMHD1 expression levels (four groups). The curves are colored by different SAMHD1 expression levels: blue (1): ≤ 0.5 ; green (2): > 0.5 and ≤ 0.67 ; gray (3): > 0.67 and ≤ 1 ; and black (4): > 1 . R-CHOP, rituximab, cyclophosphamide, doxorubicine, vincristine, prednisolone; R-DHAP, rituximab, dexamethasone, cisplatin, high-dose cytarabine; R-FC, rituximab, fludarabine, cyclophosphamide

Next, we performed a similar analysis for our subset of the MCL Elderly trial. Here, we observed that higher SAMHD1 expression was significantly associated with improved FFS in the R-CHOP arm (HR 0.26, $P = .0416$, Figure 3A, Table 2), but not in the R-FC arm (HR 1.3, $P = .64$, Figure 3B, Table 2). In accordance, we observed that the HR for the comparison of R-FC vs R-CHOP increased with increasing SAMHD1 levels (interaction $P = .014$, Supplementary Table 4).

3.5 | SAMHD1 mutations in MCL patients

SAMHD1 mutations have been reported in lymphatic malignancies.^{18,26} These mutations are mostly deleterious and were described to cause a reduction of SAMHD1 protein expression.¹⁸ To assess the influence of SAMHD1 mutations on SAMHD1 expression levels and outcome after cytarabine-based induction treatment in MCL patients, we sequenced the SAMHD1 gene locus in 182 patients treated in the

MCL Younger and Elderly trial. Thirteen patients (7.1%) harbored at least one mutation in the *SAMHD1* gene. The *SAMHD1* mutation status did not significantly affect the FFS ($P = .47$) among these patients (Supplementary Figure 3A). By adjusting for the MIPI, the HR was 1.5 ($P = .25$) for the FFS in *SAMHD1* mutated vs unmutated patients. The *SAMHD1* expression levels were available for 44 patients with known mutation status, of whom three patients harbored at least one *SAMHD1* mutation. *SAMHD1* mutated cases tend to result in a decreased *SAMHD1* protein expression, without reaching statistical significance (Supplementary Figure 3B, $P = .25$).

3.6 | Combination treatment overcomes negative impact of *SAMHD1* in vitro

Based on the results obtained above, we further hypothesized that the combination treatment used in the MCL Younger and Elderly trial reduces the prognostic value of *SAMHD1* expression. Therefore, we quantified the expression level of *SAMHD1* in MCL (Granta-519, MAVER-1, Mino, JVM-2, U-937, UPN1, Z-138), diffuse large B cell lymphoma (DLBCL) (HLY-1, OCI-Ly3, OCI-Ly19, SU-DHL-4, SU-DHL-6, DoHH2) and Burkitt lymphoma (BL) (Raji, BL-60) cell lines by western blot (Figure 4A) and intracellular flow cytometry. *SAMHD1* was heterogeneously expressed across these cell lines and both approaches showed a high correlation (Figure 4B, Pearson correlation coefficient $R = .85$, $P < .0001$). Next, we measured the viability of the abovementioned cell lines after treatment with cytarabine, fludarabine, cyclophosphamide, dexamethasone, doxorubicine, oxaliplatin and vincristine which are used in the MCL Younger or Elderly trial. We found that cell lines with high *SAMHD1* expression were less sensitive to cytarabine than cell lines with low expression levels of *SAMHD1* (Figure 4C, $R = .65$, $P = .0065$), thereby confirming results from various cancer cell lines.¹³ However, for the other chemotherapeutics, including the purine analogue fludarabine, we did not observe a significant correlation between viability after drug treatment and *SAMHD1* expression.

When combining cyclophosphamide, dexamethasone, doxorubicine, oxaliplatin, or vincristine with cytarabine, each at four different concentrations, we found that the correlation was weakened particularly by adding vincristine, and to a lesser extent also by adding oxaliplatin (Figure 4D). For doxorubicine, cyclophosphamide and dexamethasone the correlation coefficient dropped only for individual concentration levels, preferably the higher ones (Figure 4D). Supplementary Figure 4 shows the single drug response curves, illustrating that decreasing correlation coefficients are partly related to the toxicity level of the combination partner.

We observed similar tendencies when combining these drugs with fludarabine (data not shown), even though fludarabine as single agent did not reach statistical significance (Figure 4C).

4 | DISCUSSION

SAMHD1 protein expression has been shown to serve as biomarker for the response to cytarabine in consolidation treatment of AML

patients.^{10,12} Here, we report the impact of *SAMHD1* protein expression and mutation status on MCL patients of the MCL Younger⁴ and Elderly trials.⁵

We determined the *SAMHD1* protein expression in 189 MCL patients, who were treated within the MCL Younger⁴ or Elderly trials.⁵ We found that *SAMHD1* expression was not associated with the CR rate or with FFS in patients treated with high-dose cytarabine- or fludarabine-containing regimes. These results are in contrast to both abovementioned studies in AML patients. It is important to note that these studies focused on patients who received consolidation treatment with single agent high-dose cytarabine.^{10,12} However, if the authors investigated the CR rate after AML induction therapy, which consisted of cytarabine and an anthracycline, they did not observe a significant difference in *SAMHD1* expression between patients who achieved a CR and those who did not.¹⁰ Moreover, the favorable effect of low *SAMHD1* expression, was reported to be equalized during longer follow-up.¹⁵ Based on these results, the authors concluded that *SAMHD1* expression levels are less important for the efficacy of regimens which combine cytarabine with other potent chemotherapeutic drugs. Apart from high-dose cytarabine, the treatment in the MCL Younger trial included cyclophosphamide, cisplatin, vincristine, doxorubicine, dexamethasone, and the monoclonal antibody rituximab. We hypothesized that the application of cytarabine in combination with these drugs could have been a potential factor why *SAMHD1* expression levels were not predictive for the outcome of patients treated in the MCL younger trial. To corroborate this assumption, we systematically investigated combinations of these drugs with cytarabine. Although the complex pharmacodynamics of MCL combination treatments cannot be modeled in vitro, our experiments suggested that apart from cytarabine the response to neither of the MCL relevant chemotherapeutics depends on *SAMHD1* expression levels. Accordingly, the effect of *SAMHD1* expression was significantly diminished when cytarabine was combined with these drugs. Our assay did not consider that MCL patients receive myeloablative conditioning with BEAM (carmustine, etoposide, cytarabine, melphalan), which could further dilute and overcome *SAMHD1*-mediated cytarabine resistance. Though we regard this conclusion well-grounded, our study was of exploratory nature and not specifically powered to detect statistically significant differences. Therefore, we cannot definitely exclude a potential effect of *SAMHD1* expression on FFS of MCL patients.

We also evaluated the *SAMHD1* mutation status in 182 patients of the MCL Younger and MCL Elderly cohort. We found that 7.1% of patients harbored at least one *SAMHD1* mutation; however, the mutation status did not affect the FFS. Regarding that both cohorts contain only untreated patients, this mutation frequency is high compared to a study by Clifford and colleagues who found a mutation frequency of 3% in untreated CLL patients and of 11% in relapsed/refractory CLL patients; in both untreated and treated patients, *SAMHD1* was not predictive in terms of OS.¹⁸ Due to the small overlap of patients with *SAMHD1* mutation and known protein expression level, there was no statistically significant difference in protein expression levels in our MCL cohort; however, there was a trend to a lower protein

expression in *SAMHD1* mutated cases, which is in line with the abovementioned study in CLL.¹⁸

We conclude that our initial hypothesis—high *SAMHD1* expression levels would deteriorate the outcome of MCL patients treated with cytarabine-containing therapy—could not be confirmed. In synopsis with our results obtained by in vitro drug perturbation assays and the current state of knowledge, this might be due to the fact that patients of the MCL Younger trial had received high-dose cytarabine not as monotherapy but as part of a polychemotherapy regime.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICS STATEMENT

Our study was approved by the Ethics Committee of the University of Heidelberg. Informed consent was obtained in advance.

DATA AVAILABILITY STATEMENT

Data are available upon reasonable request.

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SUPPORTING INFORMATION

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

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