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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 a 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. Across both trials investigated, SAMHD1 mutations had a frequency of 7.1% (n = 13) and did not...
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

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### 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, 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 promoter methylation, was reported to be associated with Sézary syndrome\(^16\) and lung adenocarcinoma.\(^17\) Likewise, SAMHD1 mutations result in a reduced mRNA and protein expression\(^18\) 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 (MAS-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. 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$^{\text{High}}$ 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. 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. MIPI$^9$ score was included for adjustment in the analyses of the predictive value of SAMHD1 expression on FFS. P values <.05 were formally called
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 in water. Final DMSO concentrations did not exceed 0.5%. Cell viability was determined by using the ATP-based CellTiter Glo assay (Promega).
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

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**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 sub-
set (Figure 1C). We further validated our results obtained by au-
tomated 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 dis-
crete 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, includ-
ing 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
t 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 >0.7, >0.5)
for the R-CHOP only arm (Figure 2A) and the R-CHOP/R-DHAP arm (Fig-
ure 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

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

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.

### TABLE 2 Cox regression model of failure-free survival

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

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.
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
FIGURE 4  Chemotherapy combinations overcome the protective effect mediated by high SAMHD1 expression in B cell lymphoma cell lines. A. SAMHD1 expression of various B cell lymphoma cell lines (n = 16) was determined by western blot. B. Intensity values obtained by western blot were normalized and correlated with normalized median fluorescence intensity (MFI) obtained by intracellular flow cytometry. C and D. Cell lines shown in panel A were incubated with different chemotherapeutics as single compound (C) or in combination (D). C. Shown are the mean viabilities of four concentrations correlated with the corresponding normalized intensity of SAMHD1 western blot. D. The heatmaps illustrate the mean correlation coefficients ($R$) across all cell lines, as shown in panel A, but separated by each pair of concentrations. $R$ and $P$ values shown in panel B to C represent Pearson’s correlation coefficient and corresponding $P$ value.
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, \( R = .65, P = .065 \)), 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 Younger\(^{4} \) and Elderly trials.\(^{5} \)

We determined the SAMHD1 protein expression in 189 MCL patients, who were treated within the MCL Younger\(^{4} \) 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 detected 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.\textsuperscript{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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REFERENCES


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