SAMHD1 mutations in mantle cell lymphoma: disease driver conferring *in vitro* resistance to nucleoside analogues

Authors:

Marco M. Bühler[1-3], Junyan Lu[4], Sebastian Scheinost[5], Ferran Nadeu[2,6], Damien Roos-Weil[7], Manfred Hensel[8], Tharshika Thavayogarajah[9], Holger Moch[1], Markus G. Manz[9], Eugenia Haralambieva[1], Ewerton Marques Maggio[1], Sílvia Beà[2,3,6], Eva Giné[2,6,10], Elías Campo[2,3,6], Olivier A. Bernard[7], Wolfgang Huber[4,11], Thorsten Zenz[9]

Affiliations

[1]Department of Pathology and Molecular Pathology, University Hospital and University of Zurich, Zurich, Switzerland.

[2]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.

[3]Hematopathology Section, Laboratory of Pathology, Hospital Clínic de Barcelona, University of Barcelona, Barcelona, Spain.

[4]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

[5]Molecular Therapy in Hematology and Oncology, National Center for Tumor Diseases, German Cancer Research Center (DKFZ), Heidelberg, Germany.

[6]Centro de Investigación Biomédica en Red de Cancer (CIBERONC), Madrid, Spain.

[7]Gustave Roussy, INSERM U1170, Villejuif and Université Paris-Saclay Orsay, France.

[8]Mannheim Oncology Practice, Mannheim, Germany.

[9]Department of Medical Oncology and Hematology, University Hospital and University of Zurich, Zurich, Switzerland

[10]Department of Hematology, Hospital Clínic de Barcelona, University of Barcelona, Barcelona, Spain.

[11]Molecular Medicine Partnership Unit (MMPU), Heidelberg, Germany.

Corresponding author: Thorsten Zenz, Department of Medical Oncology and Hematology, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland

Email: thorsten.zenz@usz.ch

Abstract

The genomic landscape of mantle cell lymphoma (MCL) includes frequent alterations of *TP53*, *ATM*, *CCND1* and *KMT2D*. Thus far, the mutational landscape provides little information for treatment stratification. We characterized a cohort of MCL by targeted next generation sequencing and discovered *SAMHD1* as a novel recurrently mutated gene (8.5% of investigated cases, 4/47 samples). Furthermore, we provide evidence of *in vitro* resistance of *SAMHD1* mutated patient-derived MCL cells to cytarabine and fludarabine.

Introduction

Mantle cell lymphoma (MCL) is an uncommon aggressive B-cell neoplasm which predominantly affects older adults and in the majority of cases is characterized by the t(11;14)(q13;q32) IGH-*CCND1* translocation, leading to cyclin D1 overexpression. The most frequent mutations affect *ATM*, *CCND1*, *TP53* and *KMT2D* [1,2]. Although mutations provide prognostic information[3], they do not offer predictive value for treatment response with the exception of *TP53*. A recently identified MCL subtype is characterized by leukemic non-nodal disease and longer overall survival in most cases, unless high-risk genetic alterations such as *TP53* disruption occur[4]. To improve our knowledge of the mutational landscape in MCL, we used targeted next generation sequencing (NGS) and drug viability assays to explore drug susceptibility or resistance based on genomic alterations. Sterile alpha motif and histidine aspartic domain containing deoxynucleoside triphosphate triphosphohydrolase 1 (*SAMHD1*) is a gene which was first described to be mutated in Aicardi-Goutière Syndrome[5] and was later shown to also act as a HIV-1 restriction factor in dendritic and myeloid cells[6]. The known functions of SAMHD1 include regulation of the intracellular dNTP pool and DNA repair[7]. Somatic *SAMHD1* mutations have been reported to occur in lymphoproliferative disorders such as chronic lymphocytic leukemia (CLL), where mutations were found in 3% of treatment naïve and 11% of refractory/relapsed cases[8] and were detected at higher VAF after treatment[9]. Also, SAMHD1 mutations were found in T-prolymphocytic leukemia (T-PLL), in 18% of cases[10].

Methods

Detailed methods and materials are provided in the Supplement, available on the *Blood* Website. We sequenced a total of 47 MCL samples (25 leukemic and 22 nodal or extranodal) by means of targeted next generation sequencing (n=47). Whole exome (WES) and whole genome sequencing (WGS) data was available for 4 and 3 of these samples, respectively. Mutation calling was performed using a previously published pipeline[11]. To explore the prognostic significance of *SAMHD1* mutations in MCL, samples with overall survival data from our cohort (n=35) were merged with cases from a recent MCL WGS study[12] (n=55), amounting to a total of 7 *SAMHD1* mutated and

83 wild type cases. To understand the impact of mutations (including *SAMHD1*) on the response to drugs, we exposed a set of patient-derived primary MCL cells from the leukemic group (n=14, 11 *SAMHD1* wild type and 3 *SAMHD1* mutated) to increasing concentrations of cytarabine (ara-C), fludarabine, doxorubicine and nutlin-3a using the ATP-based CellTiter Glo assay (Promega), as previously described[13]. Associations between cell viability and genotype were identified by Student's t-test (two-sided, equal variance) and p-values were adjusted for multiple testing using the Benjamini-Hochberg procedure. The study was approved by the Ethics Committee Heidelberg (University of Heidelberg, Germany; S-206/2011; S-356/2013) and the Cantonal Ethics Committee of Zurich (BASEC Nr. 2018-01618). Patients provided written informed consent prior to study.

Data Sharing Statement

All identified mutations by targeted sequencing and cell viability data after drug exposure are listed in the Supplement. WES and methylation array data are available at the European Genome-phenome Archive under accession number EGAS00001001746.

Results and Discussion

In addition to mutations in known disease drivers such as *TP53* (n=19, 40.4%) and *ATM* (n=12, 25.5%), we identified mutations in *KMT2D* (n=6, 12.8%), *NFKBIE* (n=5, 10.6%) and *NOTCH1* (n=5, 10.6%) (Figure 1A). Additionally, we found *SAMHD1* mutations in four samples (n=4, 8.5%). The mutational pattern is consistent with a tumor suppressor function with nonsense, missense and frameshift mutations (Figure 1B). All *SAMHD1* mutations had a high variant allele frequency (VAF, range 87-99%), indicating hemizygous or homozygous mutations. In three of the mutated samples, genomic data revealed deletion of chromosome 20q or uniparental disomy, explaining the high VAF. The presence of SAMHD1 mutations was not associated with significant differences in overall survival (p=0.22, Figure 1C). Also, sample size of mutated cases was too small to explore associations between survival and different treatment protocols. Cell viability screening showed a significant difference in drug response to the nucleoside analogues cytarabine (adjusted p-value=1E-04) and fludarabine (adjusted p-value=4E-03), showing higher cell viability after 48 hours

in *SAMHD1* mutated compared to unmutated patient-derived MCL cells (Figure 2A), indicating that these mutations confer *in vitro* resistance. No significant differences in cell viability was observed in *TP53* mutated versus wild type samples for purine analogues, but *TP53* mutated MCL cells showed resistance for the *MDM2* inhibitor nutlin-3a (adjusted p-value 3E-03, Figure 2B).

In a recent WGS study of MCL[12], *SAMHD1* mutations were found in 5/82 cases (6.1% of samples) with two additional cases carrying *SAMHD1* deletion, thus overall at a similar rate as in our study. Cytarabine is an efficient agent in the treatment of MCL[14], and our findings support the further analysis of *SAMHD1* mutations as biomarkers of cytarabine resistance. For example, the higher incidence of *SAMHD1* mutations in refractory/relapsed CLL[8] could be due to selection of therapy resistant clones, since fludarabine is a commonly used agent (FCR regimen: fludarabine, cyclophosphamide, rituximab). We did not observe prognostic impact of *SAMHD1* mutations on outcome in our limited-sized cohort, studies with larger number of patients may be needed.
In acute myeloid leukemia (AML) decreased SAMHD1 expression has been shown to be a biomarker that positively predicts response to cytarabine[15] and decitabine[16] treatment. Furthermore, targeting SAMHD1 with the simian immunodeficiency virus (SIV) protein Vpx improved response of primary patient AML cells to cytarabine therapy[17]. Surprisingly, we found that *SAMHD1* mutations (which are predicted to impair its function) in MCL are associated with *in vitro* resistance to both cytarabine and fludarabine. SAMHD1 is expressed at lower levels in B-cells compared to T-cells and myeloid cells[18]. A disruption by mutation or deletion could lead to an imbalance in nucleotide levels ultimately resulting in cytarabine resistance, for example by increasing dCTP levels, which compete with the metabolization of cytarabine to its active form ara-CTP, since elevated dNTP levels have been demonstrated in *SAMHD1* mutated T-PLL cells[10]. The observation that AML, T-PLL and MCL with altered *SAMHD1* expression (either by deletion, mutation or other mechanisms) respond differently might therefore be explained by differences in cell type and by different functional effect depending on type of alteration. A recent article by Davenne and coworkers[19] reported functional and pharmacological effects of SAMHD1 depletion in CLL cells, notably identifying forodesine as a potent agent in cells lacking SAMHD1 and elucidating the underlying

mechanism. These results additionally raise the possibility of a targeted use of forodesine in MCL carrying disrupting *SAMHD1* mutations.

In conclusion, we report that *SAMHD1* is recurrently mutated in MCL and confers resistance to nucleoside analogue therapy *in vitro*. Further investigations are needed to potentially translate our *in vitro* result to clinical settings and to understand the resistance-conferring mechanism.

Acknowledgements

M.M.B. is supported by a research grant of the Nuovo-Soldati Foundation for Cancer Research. T.Z. is supported by the Krebsliga, the Dornonville-de-la-Cour Stiftung, the CRC and the KFSP ("Precision Haematooncology"). O.A.B. acknowledges the support of JTC 2014-143 (INCA 2016-049).

Authorship

Contribution: S.S. and D.R.W. performed the experiments; M.M.B., J.L., S.S., D.R.W., F.N., T.T., W.H., O.A.B. and T.Z. analyzed the data; H.M., M.G.M., E.H., E.M.M., S.B., E.G., M.H. and E.C. contributed critical material; M.M.B. and T.Z. wrote the manuscript; T.Z. designed the study; All authors reviewed and edited the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

References

1. Bea S, Valdes-Mas R, Navarro A, et al. Landscape of somatic mutations and clonal evolution in mantle cell lymphoma. *Proc. Natl. Acad. Sci.* 2013;110(45):18250–18255.
2. Zhang J, Jima D, Moffitt AB, et al. The genomic landscape of mantle cell lymphoma is related to the epigenetically determined chromatin state of normal B cells. *Blood*. 2014;123(19):2988–2996.
3. Ferrero S, Rossi D, Rinaldi A, et al. KMT2D mutations and TP53 disruptions are poor prognostic biomarkers in mantle cell lymphoma receiving high-dose therapy: a FIL study. *Haematologica*. 2019;haematol.2018.214056.
4. Puente XS, Jares P, Campo E. Chronic lymphocytic leukemia and mantle cell lymphoma: crossroads of genetic and microenvironment interactions. *Blood*. 2018;131(21):2283–2296.
5. Rice GI, Bond J, Asipu A, et al. Mutations involved in Aicardi-Goutières syndrome implicate SAMHD1 as regulator of the innate immune response. *Nat. Genet.* 2009;41(7):829–832.
6. Laguette N, Sobhian B, Casartelli N, et al. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature*. 2011;474(7353):654–657.
7. Daddacha W, Koyen AE, Bastien AJ, et al. SAMHD1 Promotes DNA End Resection to Facilitate DNA Repair by Homologous Recombination. *Cell Rep.* 2017;20(8):1921–1935.
8. Clifford R, Louis T, Robbe P, et al. SAMHD1 is mutated recurrently in chronic lymphocytic leukemia and is involved in response to DNA damage. *Blood*. 2014;123(7):1021–1031.
9. Amin NA, Seymour E, Saiya-Cork K, et al. A quantitative analysis of subclonal and clonal gene mutations before and after therapy in chronic lymphocytic leukemia. *Clin. Cancer Res.* 2016;22(17):4525–4535.
10. Johansson P, Klein-Hitpass L, Choidas A, et al. SAMHD1 is recurrently mutated in T-cell prolymphocytic leukemia. *Blood Cancer J.* 2018;8(1):11.
11. Nadeu F, Delgado J, Royo C, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. *Blood*. 2016;127(17):2122–2130.
12. Nadeu F, Martín-Garcia D, Clot G, et al. Genomic and epigenomic insights into the origin,

pathogenesis and clinical behavior of mantle cell lymphoma subtypes. *Blood*. In press.

13. Dietrich S, Oleś M, Lu J, et al. Drug-perturbation-based stratification of blood cancer. *J. Clin. Invest.* 2018;128(1):427–445.
14. Hermine O, Hoster E, Walewski J, et al. Addition of high-dose cytarabine to immunochemotherapy before autologous stem-cell transplantation in patients aged 65 years or younger with mantle cell lymphoma (MCL Younger): a randomised, open-label, phase 3 trial of the European Mantle Cell Lymphoma Network. *Lancet*. 2016;388(10044):565–575.
15. Schneider C, Oellerich T, Baldauf H-M, et al. SAMHD1 is a biomarker for cytarabine response and a therapeutic target in acute myeloid leukemia. *Nat. Med.* 2017;23(2):250–255.
16. Oellerich T, Schneider C, Thomas D, et al. Selective inactivation of hypomethylating agents by SAMHD1 provides a rationale for therapeutic stratification in AML. *Nat. Commun.* 2019;10(1):3475.
17. Herold N, Rudd SG, Ljungblad L, et al. Targeting SAMHD1 with the Vpx protein to improve cytarabine therapy for hematological malignancies. *Nat. Med.* 2017;23(2):256–263.
18. Schmidt S, Schenkova K, Adam T, et al. SAMHD1's protein expression profile in humans. *J. Leukoc. Biol.* 2015;98(1):5–14.
19. Davenne T, Klintman J, Sharma S, et al. SAMHD1 Limits the Efficacy of Forodesine in Leukemia by Protecting Cells against the Cytotoxicity of dGTP. *Cell Rep.* 2020;31(6):107640.

Figure Legends

Figure 1. Mutations in the analyzed mantle cell lymphoma cohort and survival estimate.

A) Gene mutations identified by targeted NGS sequencing (Oncoprint). Red cells represent mutations, top histogram indicates number of mutated genes per sample.

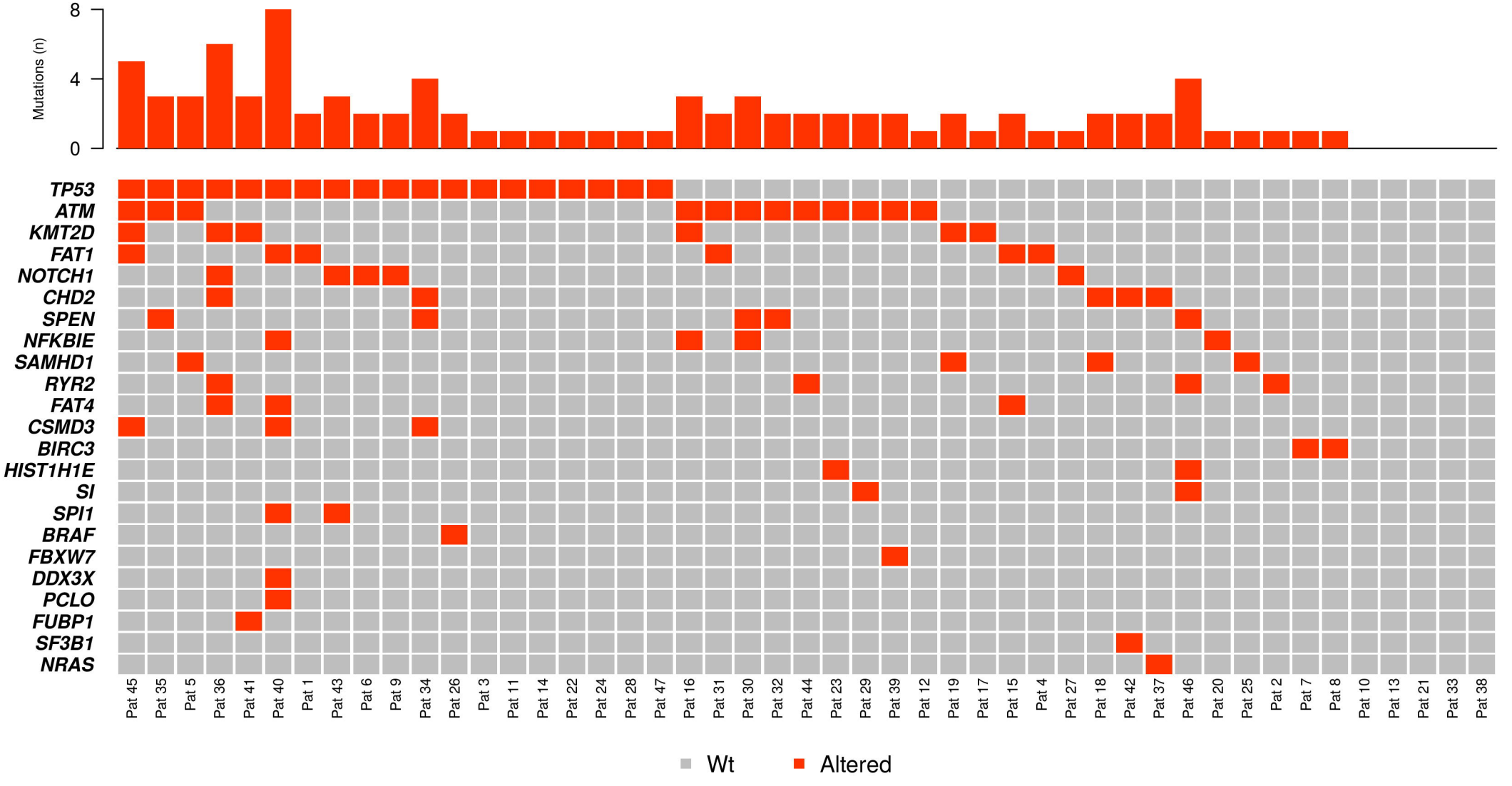
B) Schematic representation of SAMHD1 with SAM (sterile alpha motif) and HD (histidine aspartic) domain. Symbols represent found SAMHD1 mutations: nonsense (red pentagon), missense (orange circles) and frameshift (purple square) mutations.

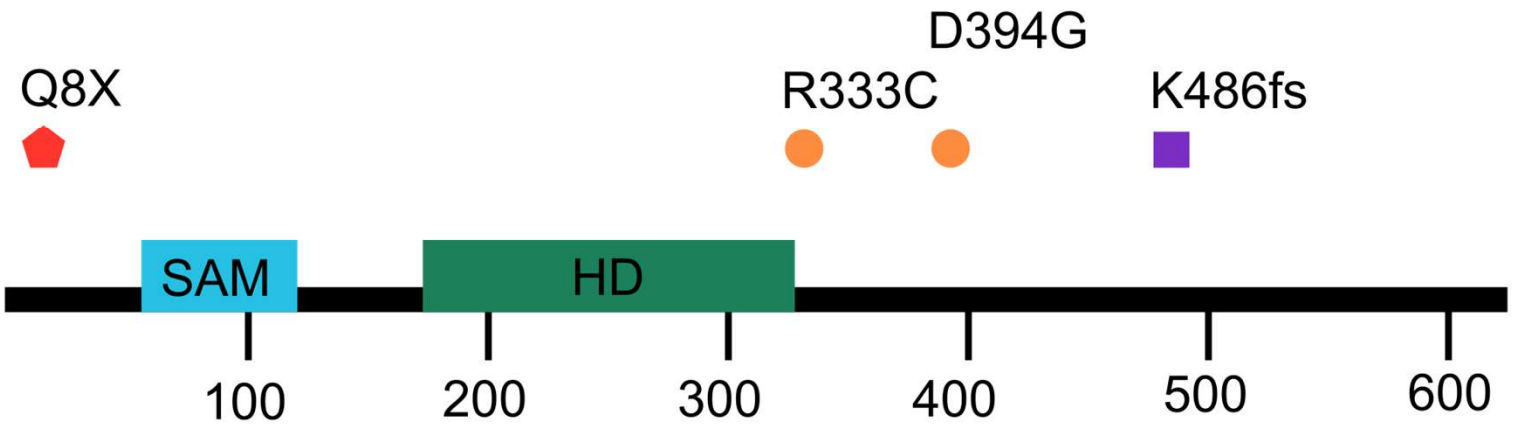
C) Kaplan-Meier estimate of overall survival of 90 MCL patients, of which seven have mutated *SAMHD1* (blue line). Censored patients are indicated by vertical marks, no significant association with survival was found (p=0.22).

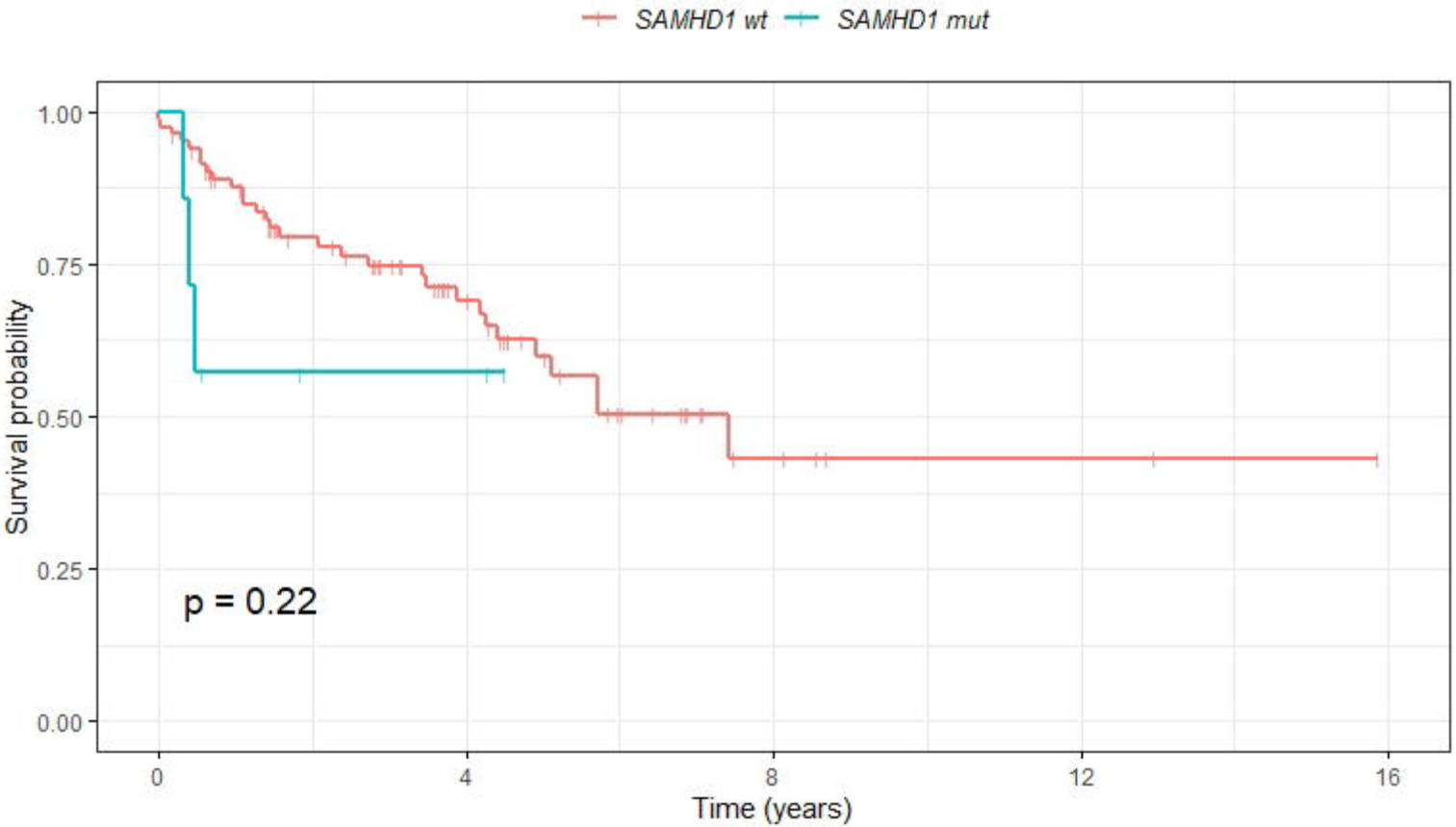
Figure 2. Effects of *SAMHD1* and *TP53* mutations on drug response.

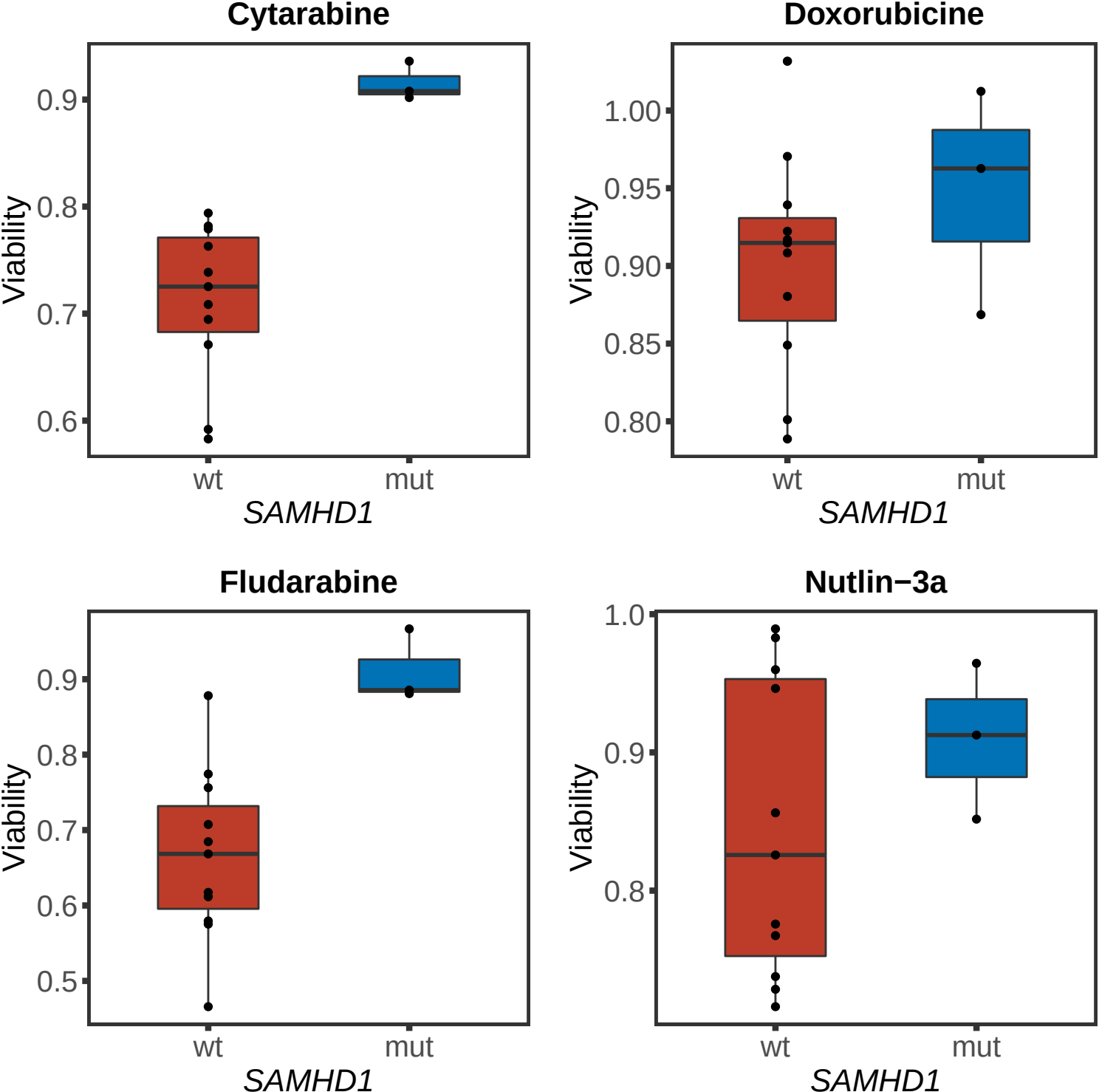
A) Boxplots illustrating cell viability after exposure of 14 MCL samples to four different drugs (cytarabine, doxorubicine, fludarabine and nutlin-3a) by *SAMHD1* mutational status. Fraction of cells alive are shown (mean of 5 concentrations tested). *SAMHD1* mutations confer resistance to fludarabine (adjusted p-value 4E-04) and cytarabine (adjusted p-value 1E-04).

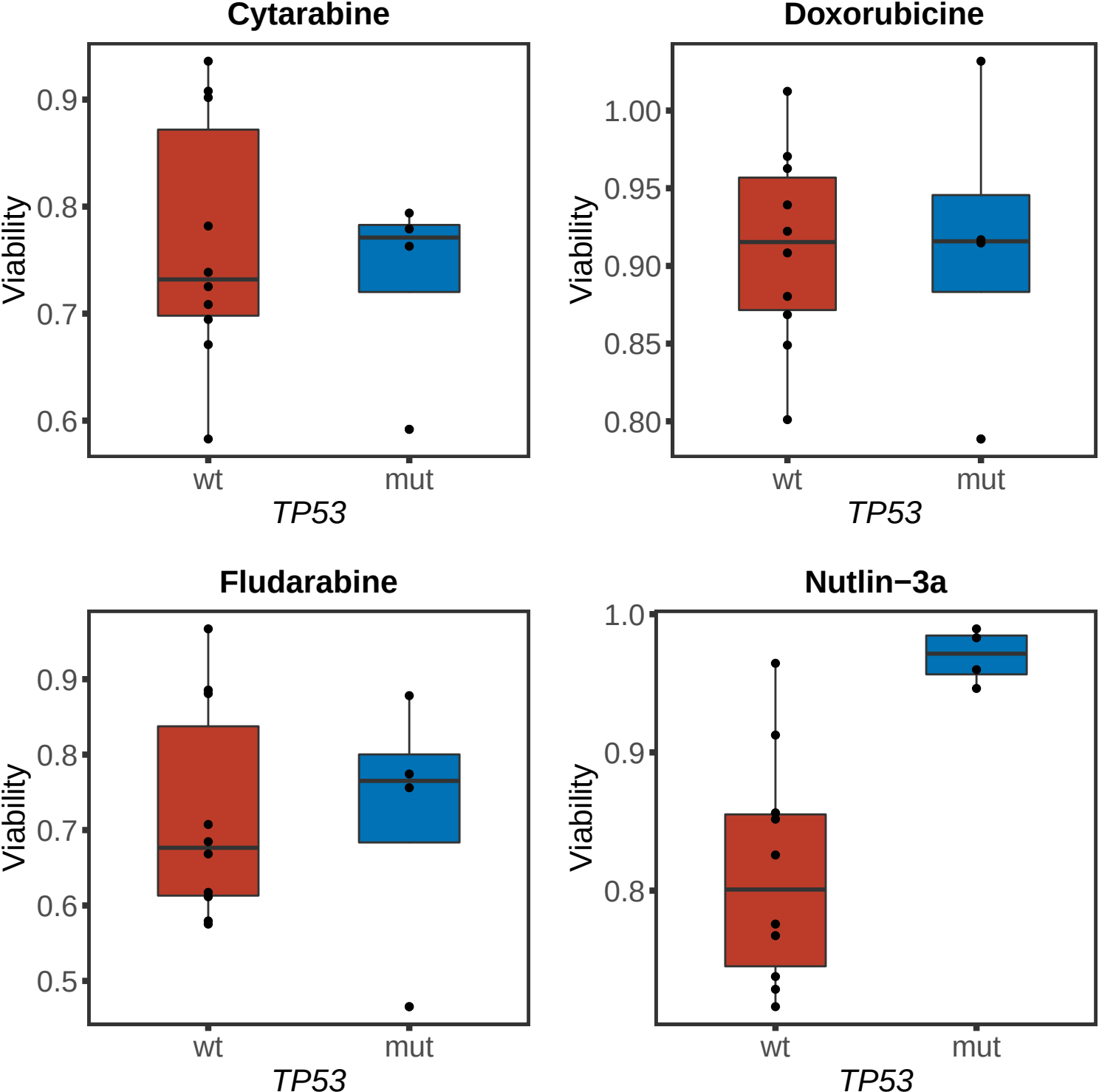
B) Boxplots illustrating cell viability after exposure of 14 MCL samples to four different drugs (cytarabine, doxorubicine, fludarabine and nutlin-3a) by *TP53* mutational status. Fraction of cells alive are shown (mean of 5 concentrations tested). *TP53* mutations confer resistance to nutlin-3a (adjusted p-value 3E-03).


Mutations (n)
8
4
0
TP53
ATM
KMT2D
FAT1
NOTCH1
CHD2
SPEN
NFKBIE
SAMHD1
RYR2
FAT4
CSMD3
BIRC3
HIST1H1E
SI
SPI1
BRAF
FBXW7
DDX3X
PCLO
FUBP1
SF3B1
NRAS
Pat 45
Pat 35
Pat 5
Pat 36
Pat 41
Pat 40
Pat 1
Pat 43
Pat 6
Pat 9
Pat 34
Pat 26
Pat 3
Pat 11
Pat 14
Pat 22
Pat 24
Pat 28
Pat 47
Pat 16
Pat 31
Pat 30
Pat 32
Pat 44
Pat 23
Pat 29
Pat 39
Pat 12
Pat 19
Pat 17
Pat 15
Pat 4
Pat 27
Pat 18
Pat 42
Pat 37
Pat 46
Pat 20
Pat 25
Pat 2
Pat 7
Pat 8
Pat 10
Pat 13
Pat 21
Pat 33
Pat 38
Wt
Altered


D394G
Q8X
R333C
K486fs
SAM
HD
100
200
300
400
500
600


SAMHD1 wt
SAMHD1 mut
1.00
0.75
0.50
0.25
0.00
Survival probability
p = 0.22
0
4
8
12
16
Time (years)


Cytarabine
Viability
0.9
0.8
0.7
0.6
wt
mut
SAMHD1
Doxorubicine
Viability
1.00
0.95
0.90
0.85
0.80
wt
mut
SAMHD1
Fludarabine
Viability
0.9
0.8
0.7
0.6
0.5
wt
mut
SAMHD1
Nutlin−3a
Viability
1.0
0.9
0.8
wt
mut
SAMHD1


Cytarabine
Viability
0.9
0.8
0.7
0.6
wt
mut
TP53
Doxorubicine
Viability
1.00
0.95
0.90
0.85
0.80
wt
mut
TP53
Fludarabine
Viability
0.9
0.8
0.7
0.6
0.5
wt
mut
TP53
Nutlin−3a
Viability
1.0
0.9
0.8
wt
mut
TP53