**SAMHD1 protects cancer cells from various nucleoside-based antimetabolites**

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**ABSTRACT**

Recently, we demonstrated that sterile α motif and HD domain containing protein 1 (SAMHD1) is a major barrier in acute myelogenous leukemia (AML) cells to the cytotoxicity of cytarabine (ara-C), the most important drug in AML treatment. Ara-C is intracellularly converted by the canonical dNTP synthesis pathway to ara-CTP, which serves as a substrate but not an allosteric activator of SAMHD1. Using an AML mouse model, we show here that wild type but not catalytically inactive SAMHD1 reduces ara-C treatment efficacy in vivo. Expanding the clinically relevant substrates of SAMHD1, we demonstrate that THP-1 CRISPR/Cas9 cells lacking a functional SAMHD1 gene showed increased sensitivity to the antimitabolites nelarabine, fludarabine, dacitabine, vidarabine, clofarabine, and trifluridine. Within this Extra View, we discuss and build upon both these and our previously reported findings, and propose SAMHD1 is likely active against a variety of nucleoside analog antimitabolites present in anti-cancer chemotherapies. Thus, SAMHD1 may constitute a promising target to improve a wide range of therapies for both hematological and non-haematological malignancies.

**Introduction**

Sterile α motif and histidine/aspartic acid domain containing protein 1 (SAMHD1) was identified in 2011 to restrict human immunodeficiency virus type 1 (HIV-1) in myeloid cells, a breakthrough in unravelling the, up to then, enigmatic reasons underlying the low permissivity of macrophages or dendritic cells to HIV-1 infection. 1,2 Soon after this discovery, the restriction mechanism of SAMHD1 was suggested to involve its enzymatic triphosphohydrolase activity, which was proposed to deplete intracellular deoxynucleoside triphosphate (dNTP) substrates for HIV-1 reverse transcription. 3,4 Subsequent studies showed that this activity of SAMHD1 is subjected to allosteric regulation by nucleotides, with the first allosteric site (A1) requiring a guanine nucleotide (GTP or dGTP) and the second allosteric site (A2) requiring any dNTP, which together allow formation of the catalytically active SAMHD1 tetramer (Fig. 1). Owing to its activity toward all canonical dNTPs and the elegant allosteric regulatory mechanism, SAMHD1 has been proposed to oppose ribonucleotide reductase (RNR) as a major regulator of DNA precursor pools, and a cell-cycle dependent differential activation of RNR (S-phase) and SAMHD1 (G1/G0-phase) has been suggested. 5 Underscoring a fundamental biological role for SAMHD1, germ-line mutations in the gene encoding SAMHD1 are associated with human diseases, such as the neurodegenerative and hyperinflammatory Aicardi–Goutières syndrome (AGS). 6 In addition, somatic aberrations of SAMHD1 have been found in chronic lymphocytic leukemia, 7 lung cancer, 8 and colorectal cancer. 9 More recently, we and others have identified SAMHD1 as an obstacle toward antimitabolite-based cancer therapies, 10–12 which will be the focus of this Extra View.

**SAMHD1 is a mediator of ara-C toxicity in AML cell models**

The global annual incidence of leukemia is about 350,000 cases, predominantly consisting of acute myelogenous leukemia (AML). 13 With the exception of pediatric patients, 14 only about one fifth of AML patients survive a 5-year period after diagnosis, and survival rates decrease substantially with the age at diagnosis. 15 Standard treatment of AML involves combination therapy of an anthracyline (most commonly doxorubicin or...
daunorubicin) and cytarabine (ara-C). The latter is a nucleoside analog that was first used for AML-induction therapy in 1968 and was later shown to also be very effective in postremission therapy. However, most of patients will eventually succumb to resistant diseases and relapses, and while it has been accepted that a worse clinical outcome directly correlates with a decrease in intracellular levels of the active metabolite ara-CTP, the underlying molecular reasons for this remained enigmatic. Shedding light on this, we and others have recently shown that SAMHD1 is a major determinant in dictating sensitivity of AML blasts to ara-C treatment, as the cell-active triphosphate metabolite of ara-C, ara-CTP, is a SAMHD1 substrate. In particular, RNA interference (RNAi)-mediated SAMHD1 knockdown, CRISPR/Cas9-induced SAMHD1 knockout or SAMHD1 degradation with Vpx-containing virus-like particles (VLPs) from simian immunodeficiency virus (SIV) increased sensitivity of AML cells to ara-C cytotoxicity. In particular, RNA interference (RNAi)-mediated SAMHD1 knockdown, CRISPR/Cas9-induced SAMHD1 knockout or SAMHD1 degradation with Vpx-containing virus-like particles (VLPs) from simian immunodeficiency virus (SIV) increased sensitivity of AML cells to ara-C cytotoxicity.10-12 Confirming the importance of the catalytic activity of SAMHD1, overexpression of wild type SAMHD1, but not allosteric site mutant D137N or the catalytic site mutants D311A or H233A, significantly reduced ara-C cytotoxicity.10,11 Interestingly, despite a proposed role of SAMHD1 phosphorylation in the restriction of retroviruses, we did not find any evidence for a role of the SAMHD1 phosho-site T592 in ara-C-CTP turn over, which is consistent with experimental evidence that dNTPase activity might be dispensable for HIV-1 restriction.28 SAMHD1 phosphorylation ablates tetramer-formation as well as HIV-1 restriction, however the dNTPase activity of phospho-SAMHD1 is only affected in conditions of low nucleotide levels.29 Our study also investigated the mechanism underlying ara-C cytotoxicity and showed that activation of the intra-S-phase and DNA-damage response pathways are substantially elevated in ara-C treated leukemic cells lacking SAMHD111, consistent with the established mechanism of action of ara-C.30-33 While Schneider et al. specifically looked at AML tumor cell lines, our study provides evidence that other haematological malignancies may involve SAMHD1 as a barrier to treatment efficacy and could possibly be antagonised to improve therapy.11 Similar to monocytic THP-1 cells, the cutaneous T-cell lymphoma line Hut-78, derived from a patient with Sézary syndrome, was also sensitized to ara-C treatment when SAMHD1 was depleted, and reconstitution of dNTPase-proficient SAMHD1 reduced ara-C cytotoxicity. This is also in support of a negative correlation of SAMHD1 mRNA expression and ara-C cytotoxicity in a panel of cell lines containing both myeloid and lymphoid neoplasms; hence SAMHD1s role as a modifier of ara-C toxicity is not restricted to myeloid neoplasms.

Figure 1. Intracellular conversion of cytarabine (ara-C) to ara-CTP and detoxification by SAMHD1. The schematic depicts canonical pathways for the intracellular synthesis of ara-CTP, the active metabolite of ara-C, which exerts DNA-damage and antiproliferative downstream effects by interfering with DNA synthesis. SAMHD1 is activated by binding of GTP or dGTP to allosteric site 1 (AS1), binding of a dNTP to allosteric site 2 (AS2) and binding of its substrate in the catalytic site. Ara-CTP is a substrate for SAMHD1 but not an allosteric activator. Abbreviations: CDA, cytidine deaminase; DCK, deoxyctidine kinase; NTSC2, cytosolic nucleotidase-II; DCTD, deoxyctidylate deaminase.
Mouse models confirm in vivo role of SAMHD1 dNTPase activity in reducing ara-C treatment efficacy

To address whether human AML tumor cells with differential SAMHD1 expression would respond differently to ara-C treatment, we used both a heterotopic as well as an orthotopic AML mouse model. Firstly, nude mice were subcutaneously transplanted with CRISPR/Cas9 THP-1 cell clones expressing SAMHD1 or not. Secondly, we injected CRISPR/Cas9 HL-60/iva cell clones containing or lacking a functional SAMHD1 gene, respectively, into the tail-vein of NOD/SCID mice. Lack of SAMHD1 expression dramatically increased the sensitivity of AML xenotransplants to ara-C induced toxicity, resulting in pronounced survival improvements. As mentioned above, it has been reported that restriction of retroviral infection by SAMHD1 can be uncoupled from its dNTPase activity, and thus we wanted to confirm that modulation of ara-C efficacy in vivo is dependent on the enzymatic activity of SAMHD1 and not mediated by other functions of SAMHD1. To perform in vivo structure-function analyses, we reconstituted SAMHD1 expression by lentiviral transduction and ectopically expressed either wild type or the catalytically inactive H233A mutant of SAMHD1 in HL-60/iva CRISPR/Cas9 SAMHD1<

SAMHD1 controls the therapeutic response of AML to ara-C

Targeting SAMHD1 with RNAi or Vpx-VLP treatment in patient-derived AML blasts sensitized those to ara-C-induced toxicity, although there was some donor-to-donor variability in the magnitude of sensitization. A retrospective analysis of the adult AML cohort from The Cancer Genome Atlas (TCGA), as well as the Therapeutically Applicable Research To Generate Effective Treatments (TARGET) cohort of children with AML, demonstrated that patients with lower SAMHD1 expression levels in AML blasts had better clinical outcome. Interestingly, our analyses did not show a significant difference in SAMHD1 expression in patients that achieved complete remission as compared with patients that did not. The survival advantage only became apparent later, i.e. during consolidation courses. This seemingly is a contradiction to the data presented by Schneider et al. that based their conclusions for the clinical significance of SAMHD1 upon immunohisto- and immunocytochemistry. As the therapy regimens did not differ substantially between the patient cohorts analyzed in both studies, this discrepancy might be explained by methodological differences. We assessed mRNA expression of SAMHD1 as a continuous measure, whereas Schneider et al. scored the staining intensity by eye categorically. It can be reasoned that protein levels do not necessarily correlate with mRNA abundance, while another possible confounder may be staining of patient-AML specific non-SAMHD1 epitopes due to the polyclonal nature of the antibody used by Schneider et al., however there is no reason to assume that unspecific staining would be more prevalent in AML patients that fail induction therapy. Ultimately, only the analysis of an independent patient cohort by immunohistochemistry, preferably using a more specific antibody, may resolve this apparent contradiction. This is not merely an academic discussion, as this will have large implications when considering SAMHD1 as a possible biomarker for treatment stratification. If the mRNA levels analyzed in 2 independent patient cohorts in our study are representative, dose modification (i.e., dose reduction for low SAMHD1 expressers and/or dose escalation for high SAMHD1 expressers) would only be justified in the post-remission phase. We feel that this is in line with the notion that anthracyclines contribute most to remission induction, and in line with the study by Mayer et al., which showed improved survival in AML with escalating doses of ara-C during the post-remission phase. In any case, reliable cut-off values of SAMHD1 expression applicable for

Figure 2. Overexpression of wild type but not catalytic-inactive SAMHD1 confers resistance to ara-C treatment in vivo. HL-60/iva CRISPR/Cas9 cells lacking endogenous SAMHD1 expression were transduced with a lentiviral vector encoding for HA-tagged wild type (black) or the catalytically-inactive H233A mutant (red) SAMHD1. Equal expression levels of ectopic SAMHD1 were confirmed by western blotting (a). Cells were xenotransplanted into NOD/SCID IL2R<

PP 0.0011

Clinical signs of disease (%) Days after onset of treatment

Clinical signs of disease (%) Days after onset of treatment

Clinical signs of disease (%) Days after onset of treatment

PP 0.0014

Survival (%) Days after onset of treatment

Survival (%) Days after onset of treatment

Survival (%) Days after onset of treatment
dose-adjustments would have to be determined by prospective studies specifically designed for this purpose and cannot simply be inferred from retrospective analyses. Furthermore, the therapeutic phase with most negative impact of SAMHD1 will also inform the study design of implementing putative SAMHD1 inhibitors into AML treatment protocols. In our manuscript, we suggested to explore Vpx as a biologic SAMHD1 inhibitor.11 As this protein ultimately has to be delivered to the cytoplasm/nucleoplasm, we made use of non-replicating VLPs as vehicle, whose pseudotyping with VSV-G guarantees delivery to the cytoplasmic compartment.36 In fact, Vpx-VLPs have been shown to increase transduction efficiency as a direct effect of Vpx,37,38 and Vpx has been suggested to improve the efficacy of lentiviral gene therapy.39,40 Lentiviral vectors are being tested in clinical trials, mainly for ex vivo transduction of hematopoietic stem cells to correct hemoglobinopathies and primary immunodeficiencies,41,42 for instance. To treat the systemic disease AML, in vivo delivery of Vpx would be required. Pre-clinical safety and toxicity data are available for lentiviral vectors, but studies for in vivo transduction to correct non-haematological genetic disorders are only in preparation.43 One major obstacle for Vpx therapy in AML is the efficiency of bone marrow targeting, however in vivo mouse experiments show that this barrier can be overcome.44,45 Alternative strategies to deliver Vpx to leukemia blasts are mRNA-based therapeutics,46 the use of small DNA viruses,47 liposomal packaging of Vpx protein48,49 or coupling of Vpx to antibodies targeting AML cells.50

SAMHD1 potentially bears some tumor suppressor functionality7,9,51-53 and thus transient inhibition of SAMHD1 only for the time of co-administration of ara-C or other antimetabolites would be preferable from a toxicity and safety perspective. Hence, viral transduction strategies with potential off-target effects54 might conceptually be the least favorable approach, and small molecule inhibitors would be ideal for controllable, safe, transient and cost-effective SAMHD1 inhibition. As germ-line mutations of SAMHD1 are causing autoimmune-like symptoms in AGS patients,6 it is also important to evaluate inflammatory reactions in putative SAMHD1 inhibitor trials. If present, these might even enhance anti-tumor effects e.g. due to interferon secretion. Although to date no cell-active SAMHD1 inhibitors have been reported, methodologies have been developed that can be used to screen small molecule libraries in vitro.55,56 From these and other studies, several nucleoside triphosphate-based small molecules have been reported to successfully inhibit SAMHD1 in vitro.12,55-57 and although these compounds currently lack potency, they provide starting points for rational design of SAMHD1 inhibitors.

Following the line of discussion in our recent manuscript regarding targeting SAMHD1 to improve therapy,11 one could speculate that AML patients with high SAMHD1 levels in leukemic blasts would eventually have a better prognosis than low SAMHD1 expressers due to the tumor suppressive activity of SAMHD1 in putative post-treatment residual AML cells. In any case, further studies on the role of SAMHD1 in maintaining genome stability and cell homeostasis are needed. In addition to general dNTP pool homeostasis, potential further explanations for tumor suppressor functions of SAMHD1 could be sanitation of endogenously occurring or stress-induced modified nucleotides, like dUTP58 or oxidised dNTPs,59-61 or perhaps the potential role of SAMHD1 in the DNA damage response,7 where it could be involved in fine-tuning dNTP requirements for repair synthesis.

**SAMHD1 neutralizes the cytotoxic effects of diverse nucleoside-based antimitabolites**

SAMHD1 is a promiscuous dNTPase that is regulated allosterically by dNTPs, and thus it is likely that several dNTP analogs, whether endogenously occurring or as part of antimitabolite-based therapies, will be SAMHD1 substrates and/or activators. Among the first modified nucleotides to be tested as substrates were several nucleoside reverse transcriptase inhibitors (NRTIs), and overall these were restrictive to SAMHD1-induced hydrolysis. Interestingly, SAMHD1 was reported to increase activity of these nucleoside analogs in cells by depleting the competing endogenous nucleoside triphosphates.59,62 Despite lack of activity toward NRTIs, several base-modified nucleotides were reported as SAMHD1 substrates, such as O6-methyl-dGTP and 5-methyl-2'-dCTP,63 however the biological relevance of this has yet to be examined. The development of a continuous enzyme-coupled assay for measuring the triphosphohydrolase activity of SAMHD1 enabled the investigation of potential nucleotide substrates, activators and inhibitors.55 It was in this report that activity of SAMHD1 toward the nucleoside triphosphate metabolite of clofarabine, an anti-cancer drug used to treat hematological malignancies, was demonstrated.55 Clofarabine-triphosphate was also suggested to be an allosteric activator.55 This was confirmed in our study, and we also demonstrated that SAMHD1 modulates clofarabine-induced toxicity in THP-1 and Hut-78 cells,11 however to a lesser extent than ara-C (see Fig. 3a and e for comparison). Supportive of a wider role of SAMHD1 in controlling clofarabine cytotoxicity, a significant negative correlation was observed between SAMHD1 expression and clofarabine-induced cytotoxicity in a panel of 133 haematological and lymphoid tissue-derived cell lines (Fig. 4a).

A recent study used in silico predictions to identify additional substrates of SAMHD1, and verified several of these predictions using an in vitro HPLC-based assay.12 To determine whether SAMHD1 modulates cytotoxicity of these and other compounds, we treated parental THP-1 or THP-1 CRISPR/Cas9 SAMHD1 knockout cells with a panel of these nucleoside analogs and measured cytotoxicity as described previously.11 As anticipated, THP-1 cells lacking SAMHD1 were 2 orders of magnitude more sensitive to cytarabine treatment (Fig. 3a). Similarly, SAMHD1−/− cells displayed increased sensitivity to vidarabine (Fig. 3b), nelarabine (Fig. 3c), fludarabine (Fig. 3d), decitabine (Fig. 3f) and trifluridine (Fig. 3g), indicating that triphosphate variants of these drugs could be substrates for SAMHD1. Although vidarabine is mainly used as an antiviral, it has been suggested as an antineoplastic agent,53 and trifluridine is another example of an antiviral drug that recently has been repurposed for the use in cancer treatment.64-66 In addition to a possible role for future cancer therapies, this may become interesting when considering SAMHD1 as a possible intrinsic resistance gene toward treatment of herpes
viral infections. In further support of a key role for SAMHD1 in controlling cytotoxicity of nelarabine and decitabine, significant negative correlations of SAMHD1 mRNA expression and toxicity of these drugs were observed in a panel of myeloid and lymphoid cell lines (Fig. 4b and c). We also reported that gemcitabine triphosphate and 6-thioguanine triphosphate are not substrates of SAMHD1, and accordingly, the absence of SAMHD1 did not affect toxicity of these drugs. The data presented here, together with the recent reports, suggests that SAMHD1 is a key player in reducing the efficacy of antimetabolite-based cancer therapies; further investigations will determine whether this is the case and to which extent this is relevant in clinical settings. The complex allosteric regulation of SAMHD1 in
combination with the enzymatic activity creates a scenario in which the net effect of a SAMHD1-interacting nucleoside toward SAMHD1 activity is composed to varying degrees of (i) being a substrate and/or competitive inhibitor at the catalytic site as well as (ii) an allosteric activator or inhibitor at either one or both of the allosteric sites. Eventually, a very simple strategy for choosing the right antinecabolite for a SAMHD1-positive cancer might be to select one that is not a SAMHD1-substrate.

Future perspectives—SAMHD1 and beyond

Our study suggests that targeting SAMHD1 bears the potential to improve outcome in AML therapy when combined with high-dose ara-C treatment. As shown by Hollenbaugh et al. and the present work, SAMHD1 may also have activity toward triphosphate metabolites of other drugs used against AML (in particular fludarabine and clofarabine) and drugs used against other types of cancers like nelarabine for T-lymphoblastic lymphoma and leukemia, decitabine for myelodysplastic syndrome (MDS), and trifluridine that in combination with the thymidine phosphorylase (TP) inhibitor tipiracil, is used against metastasised colorectal cancer and has been approved by the FDA and EMA. Tipiracil is a prime example of how the understanding of both anabolic and catabolic antinecabolite metabolism is driving the development of combination therapies to improve the efficacy of anti-cancer drugs. TP is a glycosyltransferase that removes the deoxyribose monophosphate from thymidine leaving thymine as second reaction product. It has been recognized that trifluridine monophosphate (tF-dTMP) is also a substrate for TP, countering the intracellular toxicity of trifluridine. Catabolic enzymes that reduce the effective concentration of antinecabolites have, however, been recognized long before. The deaminases cytosine deaminase (CDA) and deoxycytidylate deaminase (DCTD) convert dC or dCMP, respectively, and its analogs ara-C or ara-CMP as well as 5-aza-dC or 5-aza-dCMP to their uracil derivatives that have no or much lower antinecabolistic activity (Fig. 1). Indeed, CDA inhibitors like tetrahydouridine have been developed to increase both plasma (inhibiting plasma and liver CDA) and intracellular concentrations of ara-C. Another example of augmenting the efficacy of antinecabolites is the use of small molecule inhibitors targeting dUTPase, which hydrolyses dUTP and 5-FdUTP, an active metabolite of 5-FU, to their monophosphate forms, thereby limiting their toxic incorporation into nascent DNA. Hence, inhibitors of dUTPase can increase the efficacy of 5-FU. A more recent target to improve cytotoxic therapies with thiopurines has emerged by discovering that the nudix enzyme family member NUDT15 can hydrolyze triphosphates of 6-thioguanine and mercaptopurine. Hence, also in this case, combining these drugs with a NUDT15 inhibitor might improve therapy outcomes. It should, however, be added that these drugs are particularly suited for maintenance therapy strategies over longer periods of time. Hence, a careful preselection of patients that have a selective overexpression of NUDT15 in tumor cells should be aimed at when studying NUDT15 inhibitors clinically to omit drastic increases in toxicity – that even today are reasons to pause or cancel a treatment with thiopurines.

Based on substitutions of the stereoselective 2′ sugar moiety, Hollenbaugh et al. predicted nelarabine triphosphate, fludarabine triphosphate, BV-ara-UTP, floxuridine triphosphate, trifluridine triphosphate, sorivudine triphosphate and cladribine triphosphate as SAMHD1 substrates. It will be of interest to simulate interactions of other nucleotide drugs with the SAMHD1 catalytic site, possibly in an effort for rational design of competitive SAMHD1 inhibitors.

Several SAMHD1 inactivating single nucleotide polymorphisms (SNPs) have been described in AGS, some of them located close to the active site. Certain SAMHD1 SNPs were also reported for chronic lymphoblastic leukemia. In addition, a recent study associated SAMHD1 SNPs with colon cancer. While some of these SNPs have been shown to affect dNTPase function, possibly even in a dNTP-specific manner, there are, however, a few reports on SNPs published in public databases for which the effects on SAMHD1 dNTPase activity and stability are unknown. Future studies will have to investigate their role in cancer, their antagonisation of antinecabolite-based chemotherapies and their potential to resist small molecule inhibitors of SAMHD1. Considering compounds that bind to the active
site in a competitive manner, development of resistance to these inhibitors while retaining full ara-CTPase activity seems rather unlikely, but not impossible. Allosteric inhibitors, however, are conceptually more prone for resistance development as evidenced by allosteric inhibitors of HIV-1 reverse transcriptase.85

The fact that trifluridine triphosphate is a potential substrate of SAMHD1 could have important implications for SAMHD1-directed therapies that might be relevant for non-hematological solid tumors.86 It is important to gain more insight into why certain nucleoside analogs such as 5-FU, gemcitabine, and trifluridine are clinically most useful against solid tumors, whereas other analogs like ara-C, clofarabine, nelarabine, fludarabine and decitabine are almost exclusively reserved for myeloid and lymphoid neoplasms. Our preliminary analyses indicate that this discrepancy cannot be solely explained by differential SAMHD1 expression (data not shown). Whether general differences in tumor biology, e.g., differences in proliferation rates, or tumor-type specific transporters, metabolic enzymes or differentially activated damage-response pathways constitute the main reason for a relative resistance of solid tumors to various antimetabolites, remains to be investigated. We are hopeful that these kinds of studies will ultimately increase the arsenal of drugs that can be used to more efficiently target solid tumors.

Concluding remarks

In conclusion, our study along with the reports by Schneider et al. and Hollenbaugh et al. demonstrate that SAMHD1 is a key barrier to ara-C efficacy during AML consolidation therapy and that SAMHD1 may be a suitable target to enhance chemotherapy against other malignancies.10-12 The search for drugs to target and counteract SAMHD1 to improve chemotherapeutic treatments will hopefully lead to the discovery of compounds that, in combination with existing drugs, would reduce side effects and simultaneously make them more effective. A better understanding of their physiologic metabolism will hopefully pave the way for a less empirical and more rational design of future cancer treatments with antimetabolites. Personalised cytotoxic therapy of the future could select the best combinations from a plethora of antimetabolites in clinical use and tune their efficacy by selecting the right combination of accompanying inhibitors.

Methods

Plasmids and viral vectors

pCSxW encoding hemagglutinin-tagged wild type or catalytically-inactive H233A mutant SAMHD1, HIV-1 GagPol expression vector pCMV-ΔR8.91 and pMD.G (kind gift from D. Trono, School of Life Sciences, Lausanne, Switzerland) were described before.11 To generate guideRNA-resistant versions of these SAMHD1 expression plasmids we PCR-amplified the SAMHD1 cDNAs using forward primer (EcoRI) OTS1478 5’-ATG gaa ttc ATG CAG CGA GCC GAT TCC GAG CAG CCG TCA GCA CGA CCC AGA TGT GAC GAT TCA CCA AGA ACC CCC TCA AAC AC-3’ which contains non-coding nucleotide changes (underlined) and reverse primer (NotI) OTS1393 5’-GCA Tgc ggc Tgc CAT TGG GTC ATC TTT AAA AAG C-3’ and cloned the amplicon back into pCSxW- HA using EcoRI/NotI plLentiCRISPRv2 (Addgene plasmid #52961) encoding SAMHD1 specific gRNAs was described before.11 For viral vector production 293T cells were transfected according to described previously protocols.11

Generation of THP-1 SAMHD1 CRISPR/Cas9 cell lines and antibodies

The construction of THP-1 and HL-60/iva CRISPR/Cas9 single cell clones with disrupted SAMHD1 gene has been described before.11 We reconstituted expression of HA-tagged wild type or H233A mutant SAMHD1 by transduction with pCSxW derived lentiviral vectors encoding guide RNA-resistant SAMHD1 at a multiplicity of infection (MOI) of ~100. Due to this high MOI there was no need to drug-select for ectopic expression. We used a monoclonal rat anti-HA antibody 3F10 (Roche) directly coupled to horse-reddish peroxidase to detect ectopically expressed SAMHD1, mouse monoclonal anti-SAMHD1 antibody 1F9 (Abcam) or rabbit polyclonal anti-Hsp90 antibody H-114 (Santa Cruz) as loading control.

Mouse experiments

Ectopic SAMHD1 expression was achieved by lentiviral transduction of HL-60/iva CRISPR/Cas9 SAMHD1−/− cells. Ectopically expressed wild type or the catalytically-inactive H233A mutant SAMHD1 contained synonymous nucleotide substitutions to escape the guide RNAs expressed in the cell. Transduced cell bulks were xenotransplanted intravenously into NOD/SCID mice (each n = 6). Mice were treated with 50 mg·kg−1 ara-C (fl) before.11 We reconstituted expression of HA-tagged wild type or H233A mutant SAMHD1 by transduction with pCSxW derived lentiviral vectors encoding guide RNA-resistant SAMHD1 at a multiplicity of infection (MOI) of ~100. Due to this high MOI there was no need to drug-select for ectopic expression. We used a monoclonal rat anti-HA antibody 3F10 (Roche) directly coupled to horse-reddish peroxidase to detect ectopically expressed SAMHD1, mouse monoclonal anti-SAMHD1 antibody 1F9 (Abcam) or rabbit polyclonal anti-Hsp90 antibody H-114 (Santa Cruz) as loading control.

Cytotoxicity assay

To measure cytotoxicity we used the colorimetric CellTiter 96® AQQUeO One Solution Cell Proliferation Assay (MTS) (Promega) as described previously.11 Absorbance was measured using a Glomax Multi+ plate reader (Promega), and EC50 values were calculated using non-linear logistic regression analyses in Prism 6 (GraphPad Software), and statistics were performed by means of Extra-sum-of-squares F tests (**: P≤0.01; ***: P≤0.0001).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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