PNP inhibitors selectively kill cancer cells lacking SAMHD1

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ABSTRACT
Purine nucleoside phosphorylase inhibitors (PNP-Is) were developed to ablate transformed lymphocytes. However, only some patients with leukemia benefit from PNP-Is. We provide a molecular explanation: the deoxyribonucleoside triphosphate (dNTP) hydrolase SAM and HD domain-containing protein 1 (SAMHD1) prevents the accumulation of toxic dNTP levels during purine nucleoside phosphorylase inhibition. We propose PNP-Is for targeted therapy of patients with acquired SAMHD1 mutations.

Main text
SAM and HD domain-containing protein 1 (SAMHD1) was identified over a decade ago as a regulator of the innate immune response.1 Mutations in SAMHD1 are associated with abnormal type I interferon (IFN) expression and cause the hereditary encephalopathy Aicardi-Goutières syndrome (AGS). SAMHD1 is a deoxyribonucleoside triphosphohydrolase (dNTPase) and degrades deoxyribonucleoside triphosphates (dNTPs), the building blocks of DNA. SAMHD1 is best known for its role in controlling infection with human immunodeficiency virus (HIV). In some types of cells, SAMHD1 reduces the intracellular amounts of dNTPs to levels that prevent HIV from retrotranscribing its RNA genome into DNA. SAMHD1 also limits infection with other viruses, including herpesviruses and hepatitis B virus.

While working on the role of SAMHD1 in the control of viruses, our investigations took an unexpected turn. We compared cells with and without SAMHD1. In these experiments, cells were fed with deoxyribonucleosides (dNs). Cells take up dNs and subsequently convert them into dNTPs. The intention of this work was to investigate how dNTP levels influence virus replication. However, our studies led to a very different observation: cells without SAMHD1 started dying after feeding with dNs, whereas cells with SAMHD1 were unaffected.2 Amongst the four dNs, deoxyguanosine (dG) showed the highest toxicity. dG was converted intracellularly to deoxyguanosine triphosphate (dGTP) and triggered apoptosis in a variety of SAMHD1-deficient cell types and cell lines from human and mouse, but not in SAMHD1-sufficient cells. We concluded that SAMHD1 plays an important role in safeguarding cells against imbalances in dNTP levels (Figure 1).2

Acquired mutations in SAMHD1 were identified in different types of cancer, including in some patients with refractory chronic lymphocytic leukemia (CLL),3 as well as in lung and colon cancer. These mutations typically result in a loss of SAMHD1 protein expression.3 We, therefore, hypothesized that it may be possible to specifically kill cancer cells with acquired SAMHD1 mutations by disturbing their nucleotide metabolism.

Purine nucleoside phosphorylase (PNP) is an intracellular enzyme that converts dG into guanine, which is further degraded into uric acid. Mutations in the PNP gene cause a rare immunodeficiency characterized by reduced numbers of T lymphocytes. In 2001, Kicska et al. developed immucillin H, now better known as forodesine, as a small molecule PNP inhibitor (PNP-I).4 The intention of the authors was to kill lymphocytes by elevating intracellular dG and consequently dGTP levels and to thereby eliminate leukemic cells. Forodesine was subsequently tested in clinical trials and was found to be highly beneficial, but only in a subset of patients. This observation has thus far lacked an explanation.

Since SAMHD1 is mutated in some patients with CLL, we asked whether forodesine would specifically kill SAMHD1-deficient leukemic cells. To test this, we used peripheral blood mononuclear cells (PBMCs) from healthy volunteers and from patients with CLL with and without acquired SAMHD1 mutations. We exposed PBMCs ex vivo to forodesine and a low concentration of dG to mimic dG levels observed in the plasma of forodesine-treated patients. Using cell viability analysis and mass cytometry, we found that leukemic cells from patients with SAMHD1 loss-of-function mutations were killed by forodesine and dG (Figure 1). In contrast, normal PBMCs and leukemic cells with SAMHD1 survived.

We, therefore, propose that SAMHD1 status explains why only some patients benefit from forodesine treatment. We hope that future clinical trials will confirm this, and that PNP-Is may be developed as a precision medicine. Although PNP inhibition was initially conceived as a strategy to target leukemia, we believe that solid tumors without SAMHD1 expression may also be susceptible to this treatment. It would also be interesting to explore whether the provision of dG together with PNP-Is enhances the induction of cell death in SAMHD1-deficient malignancies. Given the short half-life of dG in

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plasma, derivatives should be considered. In another study, we recently showed that dG is sensed by Toll-like receptor 7, which induces pro-inflammatory cytokines.\(^5\) dG treatment may therefore not only kill SAMHD1-deficient cells but may also induce anti-tumor immunity.

Different nucleoside analogs are used as cancer drugs. One such example is cytarabine that is important clinically in the treatment of acute myeloid leukemia (AML). Others recently identified SAMHD1 as a biomarker in AML for the response to cytarabine.\(^6\,^7\) These studies show that SAMHD1 – in addition to its natural dNTP substrates – also degrades ara-C triphosphate (ara-CTP), which is generated intracellularly from cytarabine and causes toxicity. High expression of SAMHD1 in AML cells correlates with poor responses to cytarabine due to the degradation of ara-CTP by SAMHD1.\(^6\,^7\) Subsequent work showed that SAMHD1 also protects cancer cells against other nucleoside-based compounds, including the DNA hypomethylating agent decitabine.\(^8\,^9\)

These observations and our work highlight the potential utility of SAMHD1 inhibitors, which we predict to sensitize SAMHD1-sufficient cancer cells to PNP-Is, cytarabine and other compounds. Interestingly, efforts to find a SAMHD1 inhibitor led to the discovery that ribonucleotide reductase (RNR) inhibitors sensitize cells to cytarabine.\(^10\) RNR is involved in the intracellular synthesis of dNTPs. By disturbing the relative concentrations of dNTPs, RNR inhibition indirectly blocks the enzymatic activity of SAMHD1, which requires dNTPs as allosteric activators.\(^10\)

Taken together, our work and many other elegant studies reveal an important role of SAMHD1 as a barrier to multiple cancer treatments. Future investigations of SAMHD1 in the context of malignant disease are therefore warranted and

![Diagram of SAM and HD domain-containing protein 1 (SAMHD1)-deficient cells](image-url)

**Figure 1.** SAM and HD domain-containing protein 1 (SAMHD1)-deficient cells are susceptible to cell death triggered by deoxyribonucleoside triphosphate (dNTP) imbalances. dNTP imbalances can be induced experimentally by deoxyguanosine (dG) feeding of cells, which results in deoxyguanosine triphosphate (dGTP) buildup in cells lacking SAMHD1 (top row). Alternatively, dGTP overload and cell death can be achieved in cells without SAMHD1 by inhibition of Purine nucleoside phosphorylase (PNP) with compounds such as forodesine (bottom row). PNP inhibitors prevent degradation on dG that is converted into dGTP, which accumulates to toxic levels in the absence of SAMHD1.
should address the molecular underpinnings of how SAMHD1 modulates the effects of cancer drugs, the development of SAMHD1 inhibitors and how the acquisition of SAMHD1 mutations provides an advantage for cancer cells.

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