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TRANSLATIONAL STUDIES ON ANTIMETABOLIC THERAPIES IN PAEDIATRIC ONCOLOGY

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Karolinska Institutet

Stockholm 2023

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2023

ISBN 978-91-8017-078-9
Cover illustration: Cover designed by Henrik Lysell, "Sensitisation to treatment"

Translational studies on antimetabolic therapies in paediatric oncology

Thesis for Doctoral Degree (Ph.D.)

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The thesis will be defended in public at Inghesalen, floor 2, Tomtebodavägen 18A, Solna, Friday, September 8th, 2023, at 09:00

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To my family, who always believed in me,

and to my friends, who were always there for me

Popular science summary of the thesis

Despite often being lumped together as one disease, cancer is in fact an umbrella term for many different diseases. It can affect blood cells as well as solid organs in the body. One key feature of cancer is uncontrollable cell growth, which requires duplication of the cells. In order to replicate themselves, cancer cells, like other cells, must copy their material. Among the things they duplicate is the genetic information of the cell, which is stored in the DNA. Once the genetic material has been copied, cells then split themselves once, to create two cells from one. Each new cell has an identical copy of the original cell's DNA. As cancer cells multiply so quickly, mistakes can and do happen as they replicate their DNA.

Genetic information in the DNA is stored in the form of a sequence of four different building blocks. Human DNA exists as a double strand, which must be separated, and then each strand will be copied by adding a new complementary strand during replication. The four building blocks of DNA (the deoxyribonucleotides of adenine, guanine, thymine and cytosine) each have only one other building block that they can bind to, creating exact pairs in the sequence that must be read and copied correctly to avoid mistakes. The building blocks enter the newly synthesised strand of DNA in line as it gets longer, and in order for them to be able to be added, they need to go through a procedure called phosphorylation, where in three sequential steps, three phospho-groups will be added to them.

Since cancer cell division occurs at a faster rate than in normal cells, mismatches of these building blocks occur more often. This can cause the so-called DNA mutations and leads to production of new cells with mistakes in the DNA that often promote more unchecked growth by the cancer cells. Because of this, cells employ several safety mechanisms to ensure proper DNA replication.

One such mechanism is a protein called SAMHD1. This protein can recognise when cells do not need to duplicate and lowers the levels of the available building blocks by removing the three phospho-groups. This action prevents them from being added to the DNA, and if the DNA won't double, then the cell will not. In many different types of cancer, it has been discovered that SAMHD1 does not function as it is supposed to, presenting its importance as a guardian of this process.

One major group of drugs used for treatment in many cancer types are analogues of the 4 normal building blocks. They have a similar, but not identical structure as the normal ones, and they can follow the same procedure of phosphorylation as mentioned before and be added to the DNA. Once incorporated, however, these pseudo-building blocks perturb replication which can lead to cell death. Since they share similarities with the physiological building blocks, they too can be recognized by SAMHD1 to remove the

phospho-groups and stop their incorporation. Not all cells express the same proteins and not all cells express them at the same levels. Thus, high levels of SAMHD1 may inhibit effective treatment in cancer types where these kinds of drugs are used.

With the present thesis, we discovered that patients with acute myeloid leukaemia, a type of blood cancer, undergoing treatment with the building block analogue cytarabine, fared better when they had low levels of SAMHD1. We also found a group of drugs that can act against SAMHD1 if it is present in high levels, leading to improved effect of cytarabine when used in combination. The combination of a SAMHD1-inhibitor with cytarabine improved outcome in patients with acute myeloid leukaemia. In addition, we found that in a different type of blood cancer (called T-lymphoblastic leukaemia) treated with the analogue nelarabine, SAMHD1 could block its toxicity and thus reduce its antileukemic effects. Therefore, combining SAMHD1-inhibition with certain types of traditional chemotherapy shows promising results and can help improve patient treatment and outcome.

Abstract

Cure rates for paediatric and adult cancer patients have improved within the last decades. This can partly be explained by implementation of new technologies and methodologies such as the identification of new mutations after sequencing that can be directly targeted for treatment or the introduction of immunotherapy. However, there is an urgent need for improvement of survival particularly for patients with relapsed metastatic disease.

More than 20 years ago, SAMHD1 was discovered and even though its key role in preventing viral HIV-1 infections was initially established, it was only later classified as the first deoxynucleoside triphosphate triphosphohydrolase that can remove the three phosphogroups of the dNTPs in a single reaction, which contributes to the dNTP pool homoeostasis by limiting potentially hazardous expansion of the intracellular dNTP pool. SAMHD1 is a homotetramer that is strictly regulated by the dNTP levels, with two allosteric sites (AS1 and AS2) and one catalytic site responsible for the dNTPase activity.

Cancer cells are, among other hallmarks, characterized by loss of proliferation inhibition. It is therefore not surprising that in many cancer types, deregulation, or mutations of *SAMHD1* have been reported that allow cells to circumvent dNTP shortage to permit further DNA replication. Many chemotherapeutic drugs target uncontrolled cancer proliferation. For example, a large group of these compounds are analogues of physiological nucleosides leading to inhibition of DNA replication. SAMHD1 has the capacity to use many of these analogues as substrates and through its dNTPase activity, it dephosphorylates them and prevents their incorporation into the nascent DNA chain. This can lead to treatment resistance effectively inactivating chemotherapy. One of these analogues frequently used in regimens against haematological malignancies is cytarabine. However, its active metabolite ara-CTP is a substrate for SAMHD1, hence SAMHD1-positive cancers might limit its cytotoxic efficacy. Therefore, SAMHD1 represents a promising therapeutic target, and its inhibition might enhance cytarabine efficacy.

In the present thesis, we aimed to investigate whether there is an association between SAMHD1 expression and response to treatment with nucleoside analogues in two different haematological malignancies and whether SAMHD1 inhibition can improve current treatment protocols.

In **paper I**, we performed a phenotypic screen of more than 33000 small molecules and discovered non-competitive inhibitors of ribonucleotide reductase to potentiate cytarabine in a SAMHD1-dependent manner. Inhibition of SAMHD1 activity towards ara-CTP occurred in an indirect manner as RNR inhibition led to dNTP ratio imbalances affecting SAMHD1 substrate specificity. As dCTP outcompeted dATP as dominant AS2 activator, SAMHD1 activity towards ara-CTP was gradually lost. Functionally, the RNR

inhibitors hydroxyurea or gemcitabine acted synergistically with cytarabine, and sensitized cells to treatment in a SAMHD1-dependent manner, both in cell lines and in patient derived AML blasts. Furthermore, combination treatment prolonged survival in murine AML models. As a result, with this study we discovered already clinically available drugs that could act synergistically with cytarabine and improve treatment outcome. Hence, SAMHD1 can act as a biomarker for AML patients and combining cytarabine with RNR inhibitors might overcome SAMHD1-mediated resistance.

In **paper II**, we showed that another nucleoside analogue, nelarabine, that is specifically cytotoxic against malignant T-cells was both an allosteric activator and a substrate for SAMHD1, thus limiting its cytotoxic efficacy. SAMHD1 depletion led to treatment sensitization and addition of hydroxyurea in SAMHD1 expressing cells, inhibited SAMHD1 catalytic activity and increased intracellular levels of the active metabolite ara-GTP. Finally, in T-ALL patient derived cells, addition of HU improved the efficacy of nelarabine treatment. All in all, we showed that SAMHD1 expression is a resistance factor in nelarabine treatment and inhibition with HU could have a potential clinical use.

In **paper III**, based on our preclinical data we performed a small phase 1 clinical trial to validate the efficacy and safety of adding hydroxyurea to cytarabine-based treatment of AML patients. A total of nine patients were enrolled and they received a minimum of two cycles of treatment including daunorubicin, cytarabine and hydroxyurea. Analysis of blood mononuclear cells of patients showed that adding HU increased ara-CTP levels *in vivo*. All patients achieved complete remission (CR) without unexpected or unacceptable toxicities and MRD was negative in all eight patients that could be evaluated. Thus, CR of all patients combined with the pharmacokinetic studies, suggested that adding HU to alleviate the SAMHD1-based resistance barrier can be a rational strategy to improve treatment outcomes with cytarabine-based treatments,

In **paper IV**, we investigated the correlation between SAMHD1 expression and its impact on induction and consolidation therapy of AML. In two independent patient cohorts (n=98 and n=124), SAMHD1 protein expression levels were assessed via immunohistochemistry. SAMHD1 was differentially expressed in AML blasts but was not expressed in several physiological hematopoietic cells. Based on their SAMHD1 expression, samples were allocated to three different groups and although no effect of SAMHD1 expression was evident during induction therapy, patients with low SAMHD1 levels at diagnosis had significantly prolonged event-free and overall survival rates. Therefore, evaluation of SAMHD1 levels can serve as a prognostic marker and might stratify personalized treatment strategies including SAMHD1 inhibitors.

In summary, the results of this thesis show that SAMHD1 can be used as a prognostic biomarker for AML treated with cytarabine-based regimens and might stratify patients for enhanced treatment protocols adding the SAMHD1 inhibitor hydroxyurea to

cytarabine. SAMHD1 might have a similar role for the nucleoside analogue nelarabine in T-lymphoblastic malignancies. Hence, SAMHD1 might constitute a universal resistance factor for a group of nucleoside analogues, irrespective of the specific oncological diagnosis. Targeting SAMHD1 thus promises to improve outcomes for a large group of cancers.

List of scientific papers

I. Sean G Rudd[#], **Nikolaos Tsesmetzis[#],** Kumar Sanjiv[#], Cynthia Bj Paulin, Lakshmi Sandhow, Juliane Kutzner, Ida Hed Myrberg, Sarah S Bunten, Hanna Axelsson, Si Min Zhang, Azita Rasti, Petri Mäkelä, Si'Ana A Coggins, Sijia Tao, Sharda Suman, Rui M Branca, Georgios Mermelekas, Elisée Wiita, Sun Lee, Julian Walfridsson, Raymond F Schinazi, Baek Kim, Janne Lehtiö, Georgios Z Rassidakis, Katja Pokrovskaja Tamm, Ulrika Warpman-Berglund, Mats Heyman, Dan Grandér, Sören Lehmann, Thomas Lundbäck, Hong Qian, Jan-Inge Henter, Torsten Schaller, Thomas Helleday, Nikolas Herold
Ribonucleotide reductase inhibitors suppress SAMHD1 ara-CTPase activity enhancing cytarabine efficacy
EMBO Molecular Medicine (2020) 12: e10419

II. **Nikolaos Tsesmetzis,** Agnes L. Sorteberg, Sijia Tao, Ingrid Lilienthal, Hala Habash, Rakan Naboulsi, Magdelena Barbachowska, Miriam Yagüe-Capilla, Sean G. Rudd, Hong Qian, Georgios Z. Rassidakis, Leonie Saft, Joel Joelsson, Katja Prokovskaja Tamm, Raymond F Schinazi, Baek Kim, Torsten Schaller, David T Teachey, Nikolas Herold
SAMHD1 inhibition sensitises T-lymphoblastic acute leukaemia to nelarabine
Manuscript

III. Martin Jädersten, Ingrid Lilienthal, **Nikolaos Tsesmetzis,** Magda Lourda, Sofia Bengtzén, Anna Bohlin, Cornelia Arnroth, Tom Erkers, Brinton Seashore-Ludlow, Géraldine Giraud, Giti S Barkhordar, Sijia Tao, Linda Fogelstrand, Leonie Saft, Päivi Östling, Raymond F Schinazi, Baek Kim, Torsten Schaller, Gunnar Juliusson, Stefan Deneberg, Sören Lehmann, Georgios Z Rassidakis, Martin Höglund, Jan-Inge Henter, Nikolas Herold
Targeting SAMHD1 with hydroxyurea in first-line cytarabine-based therapy of newly diagnosed acute myeloid leukaemia: Results from the HEAT-AML trial
Journal of Internal Medicine, 2022 Dec;292(6):925-940

IV. George Z Rassidakis[#], Nikolas Herold[#], Ida Hed Myrberg[#], **Nikolaos Tsesmetzis,** Sean G Rudd, Jan-Inge Henter, Torsten Schaller, Siok-Bian Ng, Wee Joo Chng, Benedict Yan, Chin Hin Ng, Farhad Ravandi, Michael Andreeff, Hagop M Kantarjian, L Jeffrey Medeiros, Ioanna Xagoraris, Joseph D Khoury
Low-level expression of SAMHD1 in acute myeloid leukaemia (AML) blasts correlates with improved outcome upon consolidation chemotherapy with high-dose cytarabine-based regimens
Blood Cancer Journal, 2018 Oct 19;8(11):98

[#] Denotes equal first author contribution

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List of abbreviations

3-AP	3-aminopyridine-2-carboxaldehyde thiosemicarbazone (Triapine)
5-aza	5´-azacytidine
5-FU	5´-fluorouracil
ADA	Adenosine deaminase
ADP	Adenosine diphosphate
AGS	Aicardi-Goutières syndrome
AKT	Protein kinase B
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
Ara-C	Arabinoside cytosine (Cytarabine)
Ara-CTP	Arabinoside cytosine (Cytarabine) triphosphate
Ara-G	9-β-D-arabinofuranosyl guanine
ARD1	Arrest defective protein 1
ATR	Ataxia telangiectasia and Rad3 related protein
AS1	Allosteric site 1
AS2	Allosteric site 2
AUC	Area under concentration
B-ALL	B-cell acute lymphoblastic leukaemia
BC	Breast cancer
BCP-ALL	B-cell precursor acute lymphoblastic leukaemia
BM	Bone marrow
CAR	Chimeric Antigen Receptor
CC	Colon cancer
CCLE	Cancer cell line encyclopaedia
CD	Cluster of differentiation
CDK	Cyclin-dependent kinase
CDKN1A	Cyclin-dependent kinase inhibitor 1A
CETSA	Cellular thermal shift assay

CHK1	Checkpoint kinase 1
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CNDAC	2'-cyano 2'-deoxy 1 - (beta-D-arabinofuranosyl) cytosine
CNS	Central nervous system
CNT	Concentrative nucleotide transporter
Co-IP	Co-immunoprecipitation
CO_2	Carbon dioxide
COSMIC	Catalogue of somatic mutations in cancer
CpG	5´- C - phosphate - G - 3´
CR	Complete remission
CRC	Colorectal carcinoma
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats / CRISPR associated protein 9
CRL	Cullin RING ubiquitin ligase
CS	Catalytic site
CTCL	Cutaneous T-cell lymphoma
CtIP	CtBP-interacting protein
CTRP	Cancer therapeutics response portal
CUL4	Cullin 4
DAC	Decitabine
dATP	Deoxyadenosine triphosphate
dC	Deoxycytidine
DC	Dendritic cell
DCAF1	DDB1 and CUL4 associated factor 1
DCIP	Dendritic cell-derived interferon-gamma induced protein
dCK	Deoxycytidine triphosphate
dCTP	Deoxycytidine kinase
DDB1	Damage specific DNA binding protein 1
dF-dC	Difluodeoxycytydine (Gemcitabine)

dG	Deoxyguanosine
dGK	Deoxyguanosine kinase
dGTP	Deoxyguanosine triphosphate
DMSO	Dimethyl sulfoxide
dN	Deoxynucleoside
DNA	Deoxyribonucleic acid
dNMP	Deoxyribonucleoside monophosphate
dNTP	Deoxyribonucleoside triphosphate
dNTPase	Deoxynucleoside triphosphate triphosphohydrolase
DSB	DNA double strand breaks
dsDNA	Double stranded DNA
dsRNA	Double stranded RNA
DSS	Disease specific survival
dTTP	Deoxythymidine triphosphate
DUB	Deubiquitination enzyme
dUTP	Deoxyuridine triphosphate
EC_{50}	Half-maximal effective concentration
ECL	Enhanced chemiluminescence
EFS	Event-free survival
ENT	Equilibrative nucleotide transporter
EV71	Enterovirus 71
FBS	Foetal bovine serum
Fe	Iron
FFS	Failure-free survival
FLT3	Feline McDonough sarcoma (FMS)-like tyrosine kinase 3
GC	Gastric cancer
GM-CSF	Granulocyte-macrophage colony stimulating factor
gRNA	guide RNA
GTP	Guanosine triphosphate
HBV	Hepatitis B virus

HCC	Hepatocellular carcinoma
HDAC	High-dose cytarabine
HEAT-AML	Hydroxyurea-enhanced ara-C treatment of adult AML
HEK293	Human embryonic kidney 293 cells
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HL	Hodgkin lymphoma
HPLC	High performance liquid chromatography
HR	Homologous recombination
HRP	Horseradish peroxidase
HRS	Hodgkin and Reed Stenberg cell
HSV-1	Herpes simplex virus type 1
HTLV-1	Human T-cell lymphotropic virus type 1
HU	Hydroxyurea
IC_{50}	Half-maximal inhibitory concentration
IFI16	IFN-γ - inducible protein 16
IFN-I	Interferon type I
IFN-α	Interferon alpha
IFN-γ	Interferon gamma
IHC	Immunohistochemistry
IKZF1	IKAROS family zinc finger 1
IL	Interleukin
IMDM	Iscove's modified Dulbecco's medium
IRF3	Interferon regulatory factor 3
ISG	Interferon stimulated gene
JAK	Janus kinase
KLF4	Krüppel-like factor 4
KO	Knock out
KPNA2	Karyopherin alpha 2
KPNB1	Karyopherin beta 1

KRAS	Kirsten rat sarcoma viral oncogene homolog
LAC	Lung adenocarcinoma
LINE-1	Long-interspersed element 1
MAPK	Mitogen-activated protein kinase
mBMDM	Murine bone marrow derived macrophage
MCL	Mantle cell lymphoma
MDDC	Monocyte – derived dendritic cell
MDM	Monocyte – derived macrophages
MDS	Myelodysplastic syndrome
Mg	Magnesium
miRNA	MicroRNA
MLL	Mixed lineage leukaemia
MNC	Mononuclear cell
MRD	Minimal residue disease
MRE11	Meiotic Recombination 11
mRNA	Messenger RNA
MS	Mass spectrometry
NA	Nucleoside analogue
NAA10	N-alpha-acetyltransferase 10
NCS	Neocarzinostatin
NEDD8	Neural precursor cell expressed developmentally downregulated protein 8
NHEJ	Non-homologous end joining
NF-κB	Nuclear Factor kappa beta
NLS	Nuclear localization signal
NOD/SCID	Non-obese diabetic / severe combined immunodeficiency
NONO	Non-POU domain-containing octamer-binding protein
NRTI	Nucleoside reverse transcriptase inhibitors
NSCLC	Non-small cell lung cancer

NT	Nucleoside transporter
NTP	Nucleoside triphosphate
OC	Ovarian cancer
OS	Overall survival
PARP	Poly (ADP-ribose) polymerase
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PenStrep	Penicillin - streptomycin
PFS	Progression-free survival
Pi	Inorganic monophosphate
PI3K	Phosphoinositide 3-kinase
PMA	Phorbol-12 myristate-13 acetate
PNP	Purine nucleoside phosphorylase
PP2A	Protein phosphatase 2A
PPase	Inorganic pyrophosphatase
PPi	Inorganic pyrophosphate
PPPi	Inorganic triphosphate
PTEN	Phosphatase and tensin homolog
R/R	Relapse / Refractory
RC	Rectal carcinoma
RFS	Relapse-free survival
RNA	Ribonucleic acid
RNAi	RNA interference
RNAse	Ribonuclease
RNR	Ribonucleotide reductase
ROS	Reactive oxygen species
RPMI-1640	Roswell Park Memorial Institute 1640 medium
RT	Room temperature
RT-qPCR	Real-time quantitative PCR

RTI	Reverse transcription intermediates
SAMHD1	Sterile alpha motif (SAM) and histidine-aspartate (HD) domain containing protein 1
siRNA	Small interference RNA
SIRT1	Sirtuin 1
SIV	Simian Immunodeficiency Virus
SKCM	Skin cutaneous melanoma
SKP2	S-phase kinase-associated protein 2
SNP	Single nucleotide polymorphism
SS	Sézary syndrome
ssDNA	Single stranded DNA
ssRNA	Single stranded RNA
STAT	Signal transducer and activator of transcription
STING	Stimulator of interferon genes
SUMO	Small ubiquitin-like molecules
T-ALL	T-cell lymphoblastic leukaemia
T-PLL	T-cell prolymphocytic leukaemia
THP-1	Tohoku Hospital Paediatrics 1
TK2	Thymidine kinase 2
TKI	Tyrosine kinase inhibitor
TMA	Tissue microarray
TNF-α	Tumour necrosis factor alpha
TP	Triphosphate
TPO	Thrombopoietin
TRIM21	Tripartite motif protein 21
USB7	Ubiquitin specific protease 7
UTR	Untranslated region
VLP	Virus-like particle
Vpx	Viral protein X
ZIP	Zero-interaction potency

1 Introduction

Even though in paediatric acute myeloid leukaemia (AML) and T-cell acute lymphoblastic leukaemia (T-ALL) the cure rates have improved during the past decades, not all patients are cured, and in particular relapsed or refractory disease remains a therapeutic challenge[1,2]. Although new techniques and treatment approaches have been developed, we believe that a better understanding of nucleoside metabolism could be beneficial for future targeted therapies and could improve prognosis, as drugs interfering with nucleotide metabolism are important for the treatment of leukaemias.

Sterile alpha motif (SAM) and histidine (H)-aspartate (D) domain containing protein 1 (SAMHD1) is an enzyme involved in nucleoside metabolism. It is the first enzyme described with a deoxynucleotide triphosphate triphosphohydrolase activity that can directly affect the concentration of intracellular deoxyribonucleoside triphosphate (dNTP) pools. Mutations of SAMHD1 have been reported in many cancer types, showing the important role in dNTP regulation and the ability to prevent uncontrollable cell proliferation. Furthermore, studies have shown that SAMHD1 can use as substrates members of a large group of antimetabolites used at cancer treatment. These are called nucleoside analogues (NAs), and SAMHD1 enzymatic activity makes them inactive, thus exhibiting treatment resistance.

Through this thesis, a better overview of SAMHD1 functions will be discussed and how its ability to use NAs as substrates can alter treatment outcome. We will discuss the potential of SAMHD1 as a biomarker in different pathological conditions and how mutations and transcriptional and post-translational modifications effect its efficacy. Finally, druggable SAMHD1 inhibition and combinations with current treatment will be presented and the possibility of new targeted therapies will be described.

2 Background

2.1 Homeostasis of intracellular dNTP pools

A very important feature for the proper function of all cells is the ability to maintain a balanced intracellular dNTP pool. Sustaining the levels of the dNTPs to physiological levels through homeostasis, depending on the cell's needs and the cell cycle phase, is essential for the cell's genomic integrity and the protection against uncontrollable proliferation[3]. Nonetheless, not only regulation of the total amount of dNTPs is important for genome stability, but also the ratios among the four canonical dNTPs. Imbalances among the dNTP levels have been associated with genetic syndromes and cancer development[4]. The dNTP pools are strictly regulated, and a plethora of different enzymes are involved for the generation of dNTPs. Since cancer cells proliferate faster and rely on dNTP abundance, targeting these enzymes is proven to be an important strategy for cancer treatment[5,6]. There are two distinct pathways for the generation of dNTPs and ample feedback mechanisms couple their activity to levels and balances in the dNTP pool. One is called "*de novo* pathway", where the purine and pyrimidine rings are produced from precursor molecules, and the other one is called "salvage pathway", where nucleosides are generated from pre-existing degraded deoxyribonucleic acid (DNA) molecules[7]. One of the enzymes that has been relatively recently discovered and plays an important role in dNTP pool integrity and stability is SAMHD1.

2.2 Discovery of SAMHD1

Human SAMHD1 protein was initially described as dendritic cell-derived interferon-gamma (IFN-γ) induced protein (DCIP), which was the human homologue of mouse IFN-γ induced gene *Mg11*[8]. SAMHD1 can be upregulated by tumour necrosis factor α (TNF-α) and mediate TNF-α proinflammatory responses in lung fibroblasts. Additionally, it was also reported this protein contains a SAM and an HD domain, after which the protein was named[9]. SAM domains are present in a large number of proteins that have a diversity of functions and they are involved in protein-protein interactions with other proteins that comprise either of a SAM or a SH2 domain[10] and HD domains are involved among others in nucleic acid metabolism and have a phosphohydrolase activity[11]. Since that initial discovery many studies have scrutinized SAMHD1 expression, function and (de)regulation. It is expressed in all healthy human tissues, and the protein levels fluctuate from very high to very low depending on the tissue, largely due to the presence of SAMHD1-positive histiocytes/macrophages[12]. It should be noted that even though SAMHD1 was discovered as an interferon induced gene, the early follow up studies in human cells were not able to verify a change in SAMHD1 protein levels upon treatment with various cytokines and interferons in CD4+ T cells, monocyte-derived macrophages (MDMs) or monocyte-derived dendritic cells (MDDCs)[12,13]. However, in a human monocytic cell line named Tohoku Hospital Paediatrics 1 (THP-1), permanent depletion of SAMHD1, displayed an

increased interferon type I (IFN-I) production followed by self-induction of interferon-stimulated genes (ISGs), demonstrating the important role of SAMHD1 in controlling and restricting redundant responses of IFN-I[14].

SAMHD1 is located in the nucleus, and more specifically in the nucleoplasm and not the nucleolus and has been described as a typical non-shuttling nuclear protein[15]. The nuclear localization signal (NLS) is a ^{11}KRPR^{14} motif in its N-terminus, and absence or mutations of this motif relocalise it to the cytoplasm[16,17]. This import occurs through binding of the aforementioned localization sequence to two proteins that belong to a family of nuclear import receptors, karyopherin α2 (KPNA2) and karyopherin β1 (KPNB1)[18]. It is of great importance to note though that some of the SAMHD1 functions that will be later described, appear to be more efficacious when SAMHD1 is experimentally shuttled in the cytoplasm[19]. The importance of the HD domain has been outlined for many functions of the protein including inhibition of viral infection, dNTP pool depletion, oligomerisation, and nucleic acid binding while the SAM domain appeared to be dispensable, even though full-length protein is required for the enzyme's full efficacy[20,21].

SAMHD1 is comprised of 16 exons, however in some SAMHD1-expressing cell lines, different splice variants lacking some of these exons have been reported[22,23]. Albeit sharing some characteristics with the full-length protein, their functional properties were not clear as their half-life was shorter, and they did not show any enzymatic activity[22]. Most of the splice variants have been recorded both in healthy individuals and pathological conditions (including hepatocellular carcinoma-HCC and chronic lymphocytic leukaemia-CLL)[23,24]. Expression levels vary among the different tissues. Not only does SAMHD1 control the dNTP pools but also the relative proportions among the four individual dNTP pools. It is regulated in a cell cycle dependent manner and is mainly expressed outside of the S-phase of the cell cycle and accumulates in non-proliferating cells[25]. Silencing SAMHD1 in proliferating human fibroblasts affects cell cycle progression by disrupting the normal G_1/S phase shift, due to expanded dNTP pools[25]. SAMHD1 regulates cell proliferation most probably by pausing cell cycle progression through accumulation of cells in the G_2/M phase. Silencing of SAMHD1 also revealed that SAMHD1 knock out (KO) cells are less susceptible to apoptosis[26]. However, in immortalized cell lines in which SAMHD1 has been knocked out, differences in proliferation rates were not observed, as most probably cells adapt to the absence of SAMHD1.

2.3 Initial connection to Aicardi-Goutières syndrome

Mutations of *SAMHD1* gene were identified in 13% - 17% of patients with Aicardi-Goutières syndrome (AGS), a severe neurodegenerative disorder that mimics congenital viral infection. Almost all SAMHD1 mutants described in AGS display mislocalisation of the protein from the nucleus to the cytoplasm and loss of function[27]. SAMHD1 was implicated in immune functions, as well as cerebral vasculopathies and strokes, with the latter being

debated whether it should be considered a manifestation of AGS or a separate entity of an artery disease[28-35]. A broad range of severity regarding neurological problems can arise in patients with AGS. In a cohort of 100 children, 12% of the patients carried a *SAMHD1* mutation but compared to the different groups clustered on the basis of different genes that were mutated, SAMHD1-related patients presented mild to intermediate neurological impairment, and they could develop higher neurological abilities, including speaking and moving[36].

2.4 SAMHD1 against viral infections

2.4.1 HIV-1 infection is restricted by SAMHD1 activity, and the role of Vpx

Amongst the first characterisations of SAMHD1 functions was its ability to restrict Human Immunodeficiency Virus type 1 (HIV-1) infections, although more retroviruses have been shown to be affected by SAMHD1 activity in later studies[20,37]. Two other retroviruses; Simian Immunodeficiency Virus (SIV) and Human Immunodeficiency Virus Type 2 (HIV-2) retained the ability to infect dendritic cells (DCs) thanks to expression of Viral protein X (Vpx). Vpx is not expressed by HIV-1 and possesses an integral role for promoting viral infection in DCs. Moreover, due to its cell type preference and specificity, it was evident that the mode of action for Vpx was not based on a common viral factor transferred by infection, but instead on a cell specific component[38]. Another fact pointing towards that direction was that Vpx could counteract the proteasome-dependent restriction in viral infections and bind DCAF1 protein [damage specific DNA binding protein 1 (DDB1) and cullin 4 (CUL4) associated factor 1] to an ubiquitin ligase complex called cullin-RING ubiquitin ligase 4 (CRL4) E3, which targets proteins for degradation[39-42].

Independent studies finally discovered that the protein responsible for the ineffectiveness of HIV-1 to infect DCs as well as resting CD4+ cells was SAMHD1[43]. SAMHD1 is highly expressed in all hematopoietic cells that HIV-1 targets for infection and acts as a defence barrier against that[12]. Through its C-terminal region with enzymatic hydrolytic activity, SAMHD1 lowers the intracellular dNTP pool levels to such an extent, that reverse transcription required for viral proliferation cannot be sustained[44]. However, as shown in HeLa (but not THP-1) cells, experimentally induced excess levels of dNTPs can saturate SAMHD1 activity[26,45]. There is a correlation between HIV-1 infection efficacy and SAMHD1 expression within different subpopulations of CD4+ T cells[46]. Using an *in vitro* system where infected T-lymphocytes and MDDCs were co-cultured, high SAMHD1 protein levels in MDDCs prevented HIV-1 cell-to-cell infection[47]. Vpx can counteract SAMHD1 resistance through direct binding to it and then via ubiquitination, targeting it for proteasomal degradation[48-50]. Supporting this finding, Vpx treatment in MDM cells accelerated the proviral DNA synthesis within 8 hours after treatment[51].

2.4.2 SAMHD1 in other viral infections

Another viral defence mechanism that SAMHD1 is involved in, is against human T-cell lymphotropic virus type 1 (HTLV-1). Monocytes infected by HTVL-1 can be directed to apoptosis indirectly mediated by SAMHD1. Depletion of the dNTP pools causes an increase in reverse transcription intermediates (RTIs). This viral genetic material in turn leads to activation of a protein named STING (stimulator of interferon genes). STING interacts with the RTIs, binds to them, and triggers an apoptotic cascade[52].

In THP-1 cell line and human primary macrophages, SAMHD1 negatively controls the innate immune response to viral infections by suppressing two crucial signalling pathways, the nuclear factor kappa beta (NF-κB) and IFN-I pathways by interacting with various proteins involved in them[53]. Experiments with monocytic non-dividing cell lines, showed that the necessary function of SAMHD1 for this ability is the dNTPase hydrolytic activity and not nuclear localization. This contradicts earlier results in proliferating cells that suggested that dNTPase activity was dispensable for the inhibition of the innate immune response pathway[53,54].

2.5 Proteasomal degradation of SAMHD1

2.5.1 Vpx targets SAMHD1 to the proteasome

Even though binding occurs between the C-terminal region of SAMHD1 and the N-terminal region of Vpx, more SAMHD1 regions are required for its efficient degradation[55,56]. More specifically the presence of residue M626 is fundamental for this interaction, as well as other residues in this region are of importance, including R617, L620 and F621. Vpx initially binds to DCAF1, and this complex (DCAF1-Vpx) forms a new protein-protein interface. Residues from both DCAF1 and Vpx bind to the C-terminal region of SAMHD1, which will direct SAMHD1 for ubiquitination (**figure 1**). This interaction among Vpx with DCAF1 and SAMHD1 is necessary but not sufficient for its degradation[42,57,58]. Of note, Vpx both increases the association rate with the complex and makes the binding between SAMHD1 and the complex stronger, which could potentially be an important step regarding SAMHD1 polyubiquitination by CRL4 E3 ubiquitin ligase[59]. Human cells express two CUL4 proteins, named CUL4A and CUL4B and complexes that include either of the two proteins, have been shown to initiate SAMHD1 degradation[60]. Vpx-dependent degradation of SAMHD1 relies on a procedure called neddylation in CUL4A, one of the scaffold proteins. Indirect destabilization and disruption of the complex by inhibiting neural precursor cell expressed developmentally downregulated protein 8 (NEDD8), the responsible protein for neddylation, prevents SAMHD1 degradation, even in the presence of Vpx[61,62].

Since the Vpx binding site on SAMHD1 is adjacent to the tetramerization and catalytic sites, recruitment of SAMHD1 to the complex renders it catalytically inactive and promotes homotetramer destabilization and subsequent disassembly to dimers and monomers[57,59]. Different studies yielded conflicting results regarding SAMHD1 localization upon infection. SAMHD1 is targeted by Vpx in the nucleus and degradation occurs in the cytoplasm as it is transported there[55,63]. However, despite that Vpx-mediated ubiquitination of SAMHD1 occurs in the cytoplasm, the ubiquitinated protein is less efficiently degraded[18], so it should be hypothesised that even though SAMHD1 could be transferred at the cytoplasm for degradation, assembly of the complex still occurs in the nucleus. Finally, RNA interference (RNAi) for KPNA2 and KPNB1 nuclear transporters in MDM cells, resulted in SAMHD1 cytoplasmic localization which did not impact the efficacy of inhibiting HIV-1 infection[18].

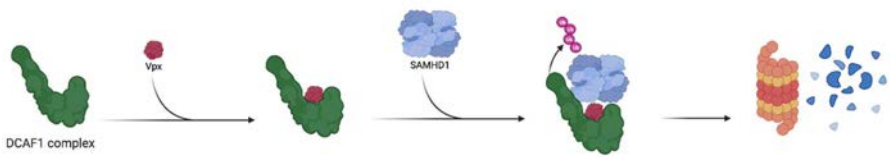

Figure 1. Vpx-dependent SAMHD1 degradation. Vpx initially binds to DCAF1 and then this complex binds to SAMHD1, leading to ubiquitination of SAMHD1 by the CRL4 E3 ligase and subsequent proteasomal degradation of SAMHD1 (*Image created with Biorender)*

2.5.2 Non-Vpx mediated degradation of SAMHD1

Apart from Vpx, other proteins expressed by the cells have been discovered to guide SAMHD1 for proteasomal degradation. This can occur either as a response to an event, for example an infection, or as a natural degrading process. In both macrophages and hematopoietic cells, cyclin L2, similar to the role of viral Vpx can bind to both DCAF1 and SAMHD1 and lead the latter for proteasomal degradation[64]. Upon enterovirus 71 (EV71) infection, it was discovered that tripartite motif protein 21 (TRIM21), an E3 ubiquitin ligase, can bind to SAMHD1 at K622 residue and induce ubiquitination and proteasomal degradation[65].

Another molecule that is associated with SAMHD1 activity and degradation is tetraspanin CD81. This discovery occurred through its role against HIV-1 infection. Among all the different members of the tetraspanin family, SAMHD1 binds only to CD81 and not to any other member. What is more interesting is that since during the cell's resting conditions SAMHD1 and CD81 are localized in different compartments, this binding should be a transient interaction. Indeed, CD81 was shown to be essential for proteasomal degradation of SAMHD1 which in turn creates an increase in dNTP concentrations. Deletion

of CD81, showed that SAMHD1 is protected from proteasomal degradation in the cytoplasm by accumulation in early endosomes[66].

Apart from directing SAMHD1 to degradation, other proteins have been reported to function in the opposite direction. An indirect rescuing mechanism was described in AML cells where non-POU domain-containing octamer-binding (NONO) protein can bind to, and prevent SAMHD1 from ubiquitination by hijacking the DDB1-DCAF1 E3 mediated proteasomal degradation[67]. A direct deubiquitination of SAMHD1 happens via direct binding of ubiquitin specific protease 7 (USP7) that belongs to the deubiquitination enzyme (DUB) family. USP7 binds at SAMHD1 and by removing the ubiquitin group from residue K421 it rescues it from proteasomal degradation[68].

2.6 Structure of SAMHD1

2.6.1 SAMHD1 structure and activation

The structure of SAMHD1 is complex and tightly regulated. Two monomers bind together to create a homodimer and then two homodimers bind together to create the catalytically active SAMHD1. Homotetramer SAMHD1 can hydrolyse dNTPs into deoxynucleosides (dNs) and inorganic triphosphate (PPPi) in a single reaction via a bi-metallic Fe-Mg centre. Absence of magnesium metal ions, destabilize the structure of the enzyme, thus impairing the interactions between the two homodimers[69]. This hydrolytic reaction can only be initiated in the presence of guanosine triphosphate (GTP) or deoxyguanosine triphosphate (dGTP)[44,70–73]. It should be noted as it will be later described, that dGTP but not GTP can be used as a substrate by the enzyme. Since intracellular levels of GTP are almost 1000 times higher than dGTP, GTP is considered the main activator of SAMHD1[72]. In the presence of all four canonical dNTPs, they can all be hydrolysed at a similar rate[71].

2.6.2 SAMHD1 is an intracellular dNTP pool regulator

Since the discovery that SAMHD1 contains an HD domain, it was speculated that this protein would act as a phosphohydrolase[11]. This hypothesis was ultimately confirmed by individual studies, recognizing SAMHD1 as a highly specific metal-dependent dNTPase (**figure 2**). It was also shown that murine SAMHD1 has the same hydrolytic and retroviral inhibition capacity as its human orthologue[74]. The dNTPase capability was also in accordance with the fact that SAMHD1 can restrict DNA proliferation of HIV-1 at the reverse transcription stage in DCs, resting CD4+ cells and MDMs, which correlates with high SAMHD1 protein levels and diminished intracellular dNTP pools[44,48,70,75]. Treating MDM cells with Vpx resulted in a rapid decrease of SAMHD1 protein levels within 4 hours and an inverse increase of intracellular dNTP pool levels at the same time[51]. Furthermore, treatment of DCs with Vpx increased all four individual dNTPs to a different extent for each species, and the total intracellular dNTP pool levels were enlarged within 24 hours

by 20-fold[13]. This agrees with other studies where Vpx treatment in MDM cells increased the dNTP pool between 5- and 33-fold, and in activated CD4+ cells between 2.5- and 7.8-fold depending on the dNTP type[44,76]. In longer observations, that were conducted 14 days post-Vpx treatment, SAMHD1 levels still remained low, at 30% expression compared to untreated MDMs. Interestingly though, as early as 5 days after treatment, dNTP levels decreased to pre-treatment levels, even with hardly detectable SAMHD1 protein, highlighting the efficacy of this enzyme in minimal levels and maybe the possibility that other enzymes might be involved in adjusting dNTP values to physiological levels[77].

Furthermore, in a xenograft immune deficient mouse model, with the use of the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats / CRISPR associated protein 9) technique, THP-1 cells either expressing or lacking SAMHD1 were injected intravenously. Remarkably, 28 days post cell injection, no difference was observed in tumour size between the two groups[78]. Mice injected with SAMHD1 KO cells, displayed longer survival, which argues that SAMHD1 expression affects survival *in vivo*, however, subcutaneous injections of the cells did not show any difference in survival rates and mice injected with KO cells presented smaller tumour formation[78-80].

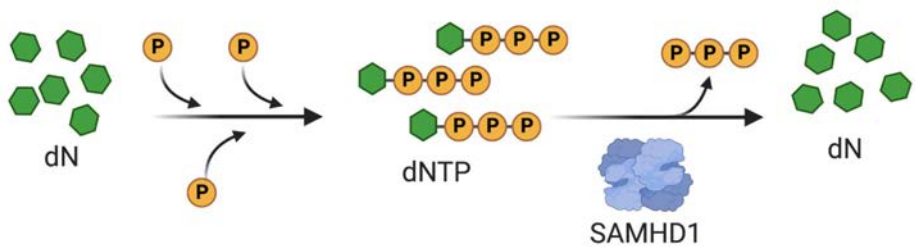

Figure 2. SAMHD1 regulation of the dNTP pool. Deoxynucleosides can be phosphorylated in three sequential steps and form the dNTPs that can be incorporated in the elongating DNA strand. SAMHD1 can regulate the intracellular dNTP pool by hydrolysing the excess dNTPs into dNs and inorganic triphosphate in a single step (*Image created with Biorender)*

2.6.3 SAMHD1 is a stable homotetramer

Structural studies initially presented that SAMHD1 is a homodimer in bacteria[70]. This discovery raised the question whether this is a shared feature in the mammalian cells. Many studies were performed, and different structures were suggested until the tetrameric form of SAMHD1 was verified. It was initially revealed to be forming oligomers that were described as complexes of higher order and that the responsible domain for this feature was the HD domain[20,81]. Eventually it was discovered that SAMHD1 is a homotetramer, with strong interactions among all four individual monomers[82]. Binding of

the individual monomers into this structural formation causes conformational changes in the enzyme and renders it a catalytically active dNTP triphosphohydrolase (**figure 3**)[82,83]. According to the very low dGTP dissociation rate from the allosteric site, it is also indicated that this structure has a very stable tertiary form[84].

In the absence of (d)GTP, SAMHD1 exists in an equilibrium between catalytically inactive monomers and dimers. It binds strongly with (d)GTP at allosteric site 1 (AS1) and creates a homodimer characterized as a "meso-stable structural entity", and the ability to form tetramers mainly depends on the availability of deoxyadenosine triphosphate (dATP)[84,85]. Except for the phosphatase capacity, SAMHD1 has been described to have more functional abilities. Homotetramer disruption due to mutations in critical sites and residues diminished the hydrolytic activity, but the (largely questioned) nuclease activity, the ability to bind to RNA and the ability to block viral infection remained operational[86,87]. Vpx-driven degradation of SAMHD1 is independent of its oligomeric state[86].

Before the discovery of the two allosteric sites in SAMHD1, it was believed that SAMHD1 required two dGTP molecules in its ASs to become active and it was speculated that one of the two could be replaced by dATP instead[82]. However, it was then discovered that SAMHD1 is a homotetramer that has two allosteric sites [allosteric site 1 (AS1) and allosteric site 2 (AS2)]. AS1 is guanine specific and can bind either GTP or dGTP but AS2 is dNTP specific and can bind any kind of dNTP but not nucleoside triphosphates (NTPs). In the absence of GTP/dGTP, the enzyme is in an equilibrium of inactive monomers and dimers. Binding of GTP/dGTP to AS1 promotes the homodimer formation, followed by a subsequent binding of any dNTP at AS2 that promotes the binding of two dimers to a homotetramer formation, which binds to any dNTP as a substrate and executes dNTP hydrolysis.

SAMHD1 can bind to all four canonical dNTPs but at different rates. Based on a biochemical assay, when GTP is bound on AS1, the combination of GTP/dATP, compared to the other dNTPs is the one that created the optimal binding conditions for the enzyme, and it created a more active enzyme. On the other hand, when dCTP is bound at AS2, the formed GTP/dCTP tetramer is inefficient in hydrolysing dCTP at the catalytic site (CS) at low dCTP concentrations. These preferences are due to changes in structural configurations that are induced by nucleotide binding. When the tetramer is formed, any one dNTP can bind the catalytic site (CS) for hydrolysis. In CS, dCTP and deoxythymidine triphosphate (dTTP) appear to be favoured, with dATP presenting the lowest affinity. Furthermore, dCTP has the highest binding affinity at AS2 and the tetramers that are induced by dCTP binding have the longest lifetime[83,88–92]. Even after the dNTP pool is depleted, SAMHD1 remains active for a long period of time, pointing out the longevity and efficacy of a very stable enzyme, the lifetime of which is subject to the dNTP bound at AS2[88,93]. As an example of this, as stated earlier, the GTP/dATP induced tetramers are the most stable and although

more than 90% of the total dATPs are hydrolysed within the first 5 minutes after the enzyme's activation, SAMHD1 can remain active for 3 hours[94].

SAMHD1 can be phosphorylated at residue T592. Phospho-SAMHD1 still has the capacity to hydrolyse dNTPs, but it is comparably less stable. When dNTP levels are high, both homotetramers exhibit effective hydrolytic activity. Contrary to that, in the presence of low dNTPs, only the unphosphorylated SAMHD1 can cleave dNTPs into dNs and PPPi. This occurs due to the conformational plasticity of the enzyme and the effect this phospho-group has on the interactions of the individual monomer and homodimer units among each other. In more detail, the impact created by this phosphorylated amino acid makes phosphorylated enzymes not able to retain a dNTP bound at AS2 comparably long as the unphosphorylated ones[94]. With the use of SAMHD1 variants carrying phosphomimetic mutations at the T592 residue, it became evident that even though the kinetics of the homotetramer formation are influenced by the phosphorylation of this threonine, the overall effect is not relatively strong to connect the retroviral restriction capacity with the enzyme's ability of tetramerization or dNTP hydrolysis[95].

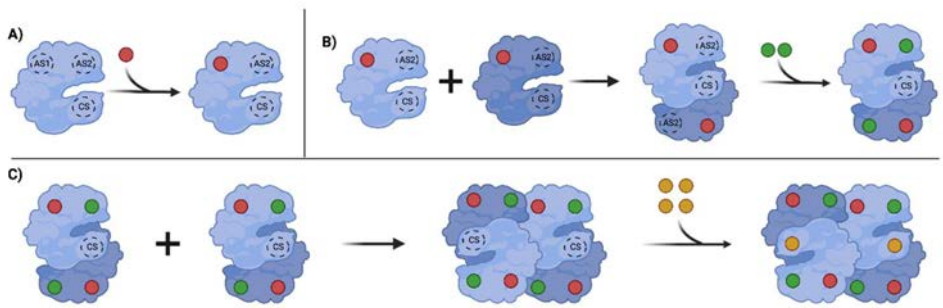

Figure 3. <u>SAMHD1 is a homotetramer.</u> Each SAMHD1 monomer can bind to three different (d)NTPs and through sequential binding can form a homotetramer. **A)** AS1 can be occupied by either GTP or dGTP, and then these monomers can bind to one dimer **B)** Any dNTP can bind to AS2 and the two dimers can bind together into forming the catalytically active homotetramer. **C)** dNTPs can bind to the CS of SAMHD1 and through the dNTPase activity, they can be cleaved into dNs and PPPi (*Image created with Biorender)*

2.6.4 Interaction with mitochondrial nucleoside metabolism

In the salvage pathway of dNTP synthesis, phosphorylation of dNs to deoxyribonucleoside monophosphates (dNMPs) can be catalysed inside the mitochondria by thymidine kinase 2 (TK2) and deoxyguanosine kinase (dGK). Moreover, hydrolase activity of SAMHD1 has also been associated with mitochondrial dNTP metabolism. When deoxyguanosine (dG) is cleaved from dGTP, it enters the mitochondria through nucleoside transporters and gets phosphorylated by dGK[96]. In normal cells unphysiological accumulation of dNTPs results in a toxic intracellular environment leading to cell death. More precisely, dG

(following phosphorylation to dGTP) is the species that confers highest toxicity at the lowest levels compared to the other dNTPs. So, presence of SAMHD1 prevents the accumulation of excess dNTPs and rescues cells from intrinsic apoptosis[97].

2.7 SAMHD1 binds to nucleic acids

One of SAMHD1 functions which remains controversial to date, is the ability to bind on nucleic acids. Studies have reported that SAMHD1 has a stronger binding preference to RNA over DNA, however, there has been contradictory evidence on which species of genetic material SAMHD1 can bind to[27,81]. Some of the studies showed that interaction and complex formation strictly occurs with single stranded RNA (ssRNA) and single stranded DNA (ssDNA) but not the double stranded ones (dsRNA and dsDNA). Besides, through analysis of SAMHD1 protein structures with mutated residues known for their importance in nucleic acid binding, it was suggested that binding to single stranded nucleic acids is important for oligomerisation[81]. Contrary to that, another group argued that binding on dsRNA could inhibit the enzyme's hydrolytic activity[20] and this inhibitory function was later supported by a another study showing that ssDNA binds on the interface of the dimer-dimer complex, thus preventing the enzyme to reach its final oligomer form[98]. However, other studies presented contradictory results as the presence of single stranded nucleic acids, did not affect GTP/dGTP binding at AS1 thus its hydrolytic activity remained unchanged[72]. Another study ultimately claimed SAMHD1 to be a ribonuclease that binds and degrades ssRNA and speculated that the main reason for all the aforementioned conflicting findings could result from different assay conditions[87].

SAMHD1 binds directly to the nucleic acids and the HD domain is likely more important for this feature while SAM domain appears to be dispensable. Furthermore, other proteins presenting nucleic acid binding properties, could also demonstrate nuclease activity, but this feature could not be verified by two independent experimental approaches[27,70]. A study of Beloglazova et al, described SAMHD1 as a metal dependent 3´→ 5´exonuclease, that cleaves one nucleotide at a time, with activity against ssDNA and ssRNA[21]. Addition of dGTP *in vitro* would inhibit the nuclease capacity of the enzyme, claiming that the dNTPase and exonuclease enzymatic processes, either function at the same protein site or dGTP binding interferes with the RNAse activity and it is the RNAse activity that regulates the HIV-1 infection restriction[21,87].

The nuclease ability of SAMHD1 was finally acknowledged as contamination from the techniques that were used, and supporting this, through using three sequential chromatography steps, the detected DNAse activity decreased by 30-fold, making it almost undetectable[99]. However, it was later argued once more by another group that using different buffers impacts the experimental outcome considerably. This group argued that SAMHD1 is a phospholytic and not hydrolytic RNAse, with preference for ssRNA and high affinity to adenine and/or uracil[100]. SAMHD1 RNAse activity was finally

reported again in a study describing that SAMHD1 has a ssRNA 3´exonuclease activity, lack of which creates RNA accumulation and facilitates into an immune response[101].

The most extensively described SAMHD1 phosphorylated residue is T592, and it is involved in many of its abilities, including highly affecting its binding efficacy in ssDNA. It appears that ssDNA binding, (d)GTP binding and polymerization, are mutually competitive functions. Binding of ssDNA can occur in monomers, regardless of their phosphorylated status, but it is easier to dissociate if not phosphorylated. Probably, since the phosphorylated SAMHD1 polymers are not as stable as their unphosphorylated counterparts, ssDNA can bind easier at the protein. So, even at the presence of (d)GTP that might bind at AS1, the rest of the ssDNA will still remain attached at the dimer-dimer interface[102].

2.8 SAMHD1 role in DNA damage repair

Fibroblasts derived from AGS patients carrying mutated SAMHD1, showed deregulation of dNTP pools, which eventually caused stress response that manifested with reduced proliferation rates and cell cycle progression arrest. This was one way to illustrate the importance of SAMHD1 activity in maintaining genome stability[103]. But besides controlling dNTPs to their physiological levels for proper cellular functions, SAMHD1 has also been discovered to play an important role in preventing cell death. In one study HeLa cells were treated with etoposide or camptothecin, two compounds that induce DNA double strand breaks (DSB). Whilst an increased rate of cell death was detected, SAMHD1 was found to be recruited to the site of damage[24]. SAMHD1 localization at the DSB sites happened upon damage. This required deacetylation at K354 residue by sirtuin 1 (SIRT1). This deacetylated SAMHD1 protein promoted homologous recombination (HR) and DSB repair predominantly in the S phase of the cell cycle through DNA end resection. Even though this acetylated residue is in proximity to AS2, the acetylation status did not affect either SAMHD1 dNTPase capacity or its ability to form homotetramers.

However, SAMHD1 acts merely as a scaffold for other nucleases and appears to have no nuclease activity by itself in this setting. SAMHD1 through the HD region of the protein, recruits the nuclease CtBP – interacting protein (CtIP), and the complex moves to DSB sites to enable DNA end resection and HR, thus promoting genome stability. This attribute is universal for all cell types, therefore targeting SAMHD1 with an inhibitor to prevent CtIP recruitment, in combination with a DSB inducing agent, might be a good strategy in promoting cell death through DNA damage[104-106]. Furthermore, USP7, a DUB binding to SAMHD1 was discovered recently. This interaction causes SAMHD1 deubiquitination and instead of being degraded, it is stabilized and relocated to DSB sites. Then upon binding with CtIP it facilitates reactive oxygen species (ROS) - induced DSB damage repair, thus giving SAMHD1 a tumour promoting role, highlighting the importance of inhibiting SAMHD1, and preventing DNA repair even more[68].

Other studies showed that SAMHD1-related cell proliferation is not only regulated by dNTP pool management, but also during S-phase. SAMHD1 is located at the same loci as the DNA replication sites and contributes to preventing ssDNA release from stalled forks. This is initiated by SAMHD1 phosphorylation by the cell cycle dependent kinases at T592 residue as will be described later. Then, phosphorylated SAMHD1 promotes fork progression by resecting and repairing DSB via direct interaction and stimulation of either CtIP or meiotic recombination 11 (MRE11) which has exonuclease activity. On top of that, through this mechanism SAMHD1 activates the ataxia telangiectasia and Rad3 related protein (ATR)-checkpoint kinase 1 (CHK1) checkpoint and therefore contributes to avoiding genomic instability[107].

Apart from HR, SAMHD1 was also revealed to participate in non-homologous end joining (NHEJ) on DNA breaks through regulation of dNTP pools by keeping them at low levels. This role was independent of the scaffolding attribute shown at HR DSB repairing. Mutations at key residues or absence of SAMHD1 caused duplications and insertions at DNA breakpoint junctions, thus producing longer intersections[108].

As mentioned earlier, a good strategy against rapidly proliferating cancer cells could be the combination of DNA damage inducing agents and SAMHD1 inhibitors. However, many cancer types develop resistance in poly(ADP-ribose) polymerase (PARP) inhibitors. PARP are enzymes with an essential role in DNA repair and inhibitors against their activity are used for cancer treatment. In one study, from one glioblastoma cell line, three resistant ones for three different PARP inhibitors were generated. All PARP-resistant cell lines presented elevated SAMHD1 protein levels and cytarabine (ara-C) resistance. This trait occurred as a response to increased DNA damage[109]. Ara-C is a NA used for treatment against many cancer types and as it will be described later, there is a direct reverse correlation between its efficacy and SAMHD1 protein levels. To sum up, increased DNA damage repair could increase SAMHD1 expression which eventually leads to diminished ara-C effectiveness.

2.9 SAMHD1-induced apoptosis

Contrary to the propensity of SAMHD1 to rescue cells by assisting in DNA damage repair, SAMHD1 expression can lead cells to apoptosis. When expressing low levels of exogenous SAMHD1 in HuT-78, a cutaneous T-cell lymphoma (CTCL) CD4+ cell line derived from a Sézary syndrome (SS) patient, cells presented cell growth inhibition and proliferation arrest. Additionally, although no immediate effect in cell cycle progression was displayed, an increased number of cells was detected in the sub-G_1 phase. This subjected cells to spontaneous apoptosis via the extrinsic pathway, in a FasL-induced manner[110]. In a different cancer setting, overexpression of SAMHD1 in an adenocarcinoma cell line, presented similar results with reduced cell proliferation rates and induced apoptosis. Cell growth delay was also showed *in vivo* confirming SAMHD1 activity as a tumour

suppressor[111]. This could be possibly explained via the hydrolytic capacity of SAMHD1, where it depletes dNTP levels to minimum levels that are not adequate for the cells to duplicate.

Using two different cell line models, THP-1 and Human embryonic kidney 293T (HEK293T), it became evident that SAMHD1 degradation in cycling cells, is very strictly regulated and it does not happen spontaneously. This controlled degradation was confirmed in three different pathways: the lysosomal, autophagosomal and proteasomal[112].

2.10 SAMHD1 regulation

2.10.1 Protein modifications

There have been many different mechanisms reported regarding SAMHD1 regulation, acting either at a transcriptional or a post-translational stage. One of these is methylation of the single CpG island in the *SAMHD1* gene promoter region, which leads to transcriptional repression. Compared to resting and differentiated CD4+ T-lymphocytes, naïve CD4+ T-cells require high levels of dNTPs for their proliferation, which is in turn achieved by downregulation SAMHD1 activity through promoter methylation[113].

Moreover, acetylation of different residues has been reported to regulate different SAMHD1 activities. Acetylation of the highly conserved residue K405 by the self-acetylated protein arrest defective protein 1 (ARD1, otherwise named as N-alpha-acetyltransferase 10, NAA10) might enhance SAMHD1 hydrolytic activity. This acetylation switch probably regulates intracellular dNTP pool quantity by decreasing it to the desired levels for promoting the G_1/S phase transition[114]. Another deacetylation mechanism of SAMHD1 takes place in residue K354 by SIRT1 and plays a pivotal role in DSB repair and HR[106].

At least three cysteine residues of SAMHD1, with the most important being C522, have been shown to be oxidized and act as an oxidation switch. Oxidation inhibits the formation of the tetramer even in the presence of dNTPs. But on the other hand, if dNTPs are already bound to the enzyme, oxidation is prevented. Another important feature of the oxidated SAMHD1 protein is the increase in the binding affinity to ssDNA by approximately 40 times, which could implicate the significance of oxidated SAMHD1 for DSB repair. While oxidated SAMHD1 is localized in the cytoplasm, it is yet still unclear if this transportation from the nucleus to the cytoplasm is a result or a prerequisite of oxidation. Oxidation at C522 occurs in a cell cycle dependent manner and through one cell cycle it can be oxidized two times, once during S-phase and once during G_2/M phase. Both of which times it is correlated to DNA repair, as replication fork repair and HR are active then[115-117].

Most modifications of SAMHD1 seem to be dependent on the cell cycle status. However, one post-translational modification of SAMHD1 that is not restricted by the proliferating

and/or differentiated stage of the cell can occur and that is SUMOylation (SUMO: small ubiquitin-like molecules). SAMHD1 can be SUMOylated at K595, a specific residue that is part of the same motif that is phosphorylated by cell cycle dependent kinases. This event, similar to T592 phosphorylation does not appear to influence the enzymatic dNTPase activity. However, even though phosphorylation and SUMOylation are two events that arise independently, and they do not regulate each other, the antiviral efficacy of SAMHD1 seems to be under modulatory control of both modification switches[118].

2.10.2 Regulation by microRNAs

Approximately, one third of the human genome has been predicted to be under regulation of micro-RNAs (miRNAs)[119]. One of them, microRNA 181 (miR-181), is an important factor regarding development, differentiation, and proliferation of various types of hematopoietic cells. It controls SAMHD1 post-transcriptionally through direct binding on the 3´-untranslated region (3´-UTR) and negatively regulates SAMHD1 protein expression[120]. Binding sites only at 3´-UTR but not the 5´-UTR or the promoter region have been associated with miRNA regulation. Likewise, in astrocytes and microglia of the central nervous system (CNS), numerous miRNAs with potential binding sites on SAMHD1 have been identified, out of which miR-155 and miR-181a (more efficiently) can downregulate *SAMHD1* expression[121]. Interferons IFN-α and IFN-γ, down-regulate miR-181a in both microglia and astrocytes (to a higher extent in astrocytes, probably due to higher baseline expression levels). Treatment with these interferons did not alter either total or phosphorylated SAMHD1 protein levels, but increased mRNA levels[122]. Another feature of IFN-α and IFN-γ is to activate the Janus kinase (JAK) / signal transducer and activator of transcription protein (STAT) pathway[123]. Treating cells with Ruxolitinib, a JAK1/2 inhibitor, followed by IFN-γ treatment, induced miR-181a expression thus decreasing *SAMHD1* expression[122].

Downregulation of SAMHD1 can also occur in CD4+ primary T-cells, an effect that can be reversed with the use of miR-181 inhibitors[124]. In primary human monocytes, both type I and type II IFNs can induce downregulation of miRNAs, which evidently leads to an increase in SAMHD1 expression levels. This regulation occurs by miR-181, miR-30 and to a lesser extent by miR-155. Also, this IFN-induced regulation is cell type specific, and does not occur in MDDCs and MDMs, and the limiting step is the lower expression of miRNAs[125].

2.11 Association with other proteins and signalling pathways

Ribonucleotide reductase (RNR) and SAMHD1 have a very clear opposite expression profile within the same cells. RNR proteins are expressed in the cytoplasm, and they are abundant within the S-phase of the cell cycle, whereas SAMHD1 is expressed in the nucleus and has its lowest expression levels during S-phase. Analogous to SAMHD1 it is regulated by the presence of NTPs and dNTPs, and it forms a heterodimeric tetramer that comprises of two different subunits named R1 and R2. Contrary to the formation of the

most stable SAMHD1 tetramers in the presence of dATP, binding of dATP at the allosteric regulatory sites of RNR have an inhibitory effect. This illustrates that the enzymatic capacity of both enzymes can be regulated by the same molecule, thus a strict regulatory mechanism governs the dNTP formation and degradation[126,127]. Treatment of MDM cells with Vpx, followed by gemcitabine (dF-dC) treatment, an RNR inhibitor, identified the contribution of RNR to dNTP pool expansion in the absence of SAMHD1[77].

In the monocytic THP-1 cell line, generation of a SAMHD1 deficient clone exhibited elevated levels of interferon type I. In addition to that, genes associated with the immune system, and cytokine or interferon signalling were also upregulated. Upregulation of interferons occurred via the phosphoinositide 3-kinase (PI3K) / protein kinase B (AKT, also known as AKT serine-threonine kinase) / interferon regulatory factor 3 (IRF3) signalling pathway, however, this characteristic appeared to be cell type specific[128]. In the same cell line, SAMHD1 can also interfere with a different downstream target of the PI3K/AKT pathway, p27, and affect cell proliferation. THP-1 SAMHD1 KO clones presented elevated phosphorylated AKT levels and downregulated phosphatase and tensin homolog (PTEN) expression, reasoning that SAMHD1 suppresses PI3K signalling pathway. Moreover, this increased pathway signalling in the KO clones demonstrated p27 mislocalisation. This protein acts as a cyclin-dependent kinase (CDK) inhibitor and arrests cell cycle at G_1, so accumulation of p27 in the cytoplasm instead of the nucleus, leads to increased cell proliferation rates[79]. Knocking out *SAMHD1* in ovarian cancer (OC) cell lines also reflected in an increase of proinflammatory cytokines and an upregulation in IFN – induced signalling[129]. Except for its function in viral infections, STING association with SAMHD1 has also been recorded in a cancer setting. In a non-small cell lung cancer (NSCLC) cell line, it was shown that SAMHD1 suppresses STING expression as a downstream target, and it can regulate and restrict lung adenocarcinoma (LAC) cancer progression. On the other hand, overexpression of STING, had a direct effect on SAMHD1-regulated cancer inhibition and instead facilitated cell growth[111]. Supporting that, depleting SAMHD1 expression in LAC cell lines, induced ssDNA fragment accumulation, leading to upregulation of IFN-γ - inducible protein 16 *(IFI16*) and subsequent STING - IFN-I pathway activation for an immune response[130].

In gastric cancer (GC) cell lines, SAMHD1 KO induced cell proliferation and clone formation while on the other hand, overexpression of *SAMHD1* had the exact opposite effects. Effects that were also presented *in vivo* in a xenograft mouse model. No differences were observed in total mouse body weight however, subcutaneously injected SAMHD1-deficient cells developed tumours bigger in size compared to the SAMHD1-proficient ones. Downregulation of the proliferation rate occurs though binding of transcription factor Krüppel-like factor 4 (KLF4) at the *SAMHD1* promoter. SAMHD1 in turn suppresses proliferation by inhibiting the mitogen - activated protein kinase (MAPK) p38 signalling

pathway via direct regulation of MAP2K6. But because in GC KLF4 is downregulated, this resulted in increased proliferation[131].

Recently, a study revealed a connection between IKAROS family zinc finger 1 (IKZF1) and SAMHD1. Generally, B-cell acute lymphoblastic leukaemia (B-ALL) lesions carrying mutations or deletions in *IKZF1* gene relate to increased relapse risk and therapy resistance. However, there is only one instance that chemotherapy outcome becomes more favourable and that is with the introduction of ara-C. Ara-C has been thoroughly described as a SAMHD1 substrate and it will be later discussed here. *SAMHD1* is under regulation of *IKZF1* and knocking out the latter, although dNTP pool expansion is promoted, also led to ara-C sensitization[132].

2.12 Phosphorylation as a regulation mechanism for SAMHD1

2.12.1 SAMHD1 phosphorylation

One of the most studied regulation mechanisms of SAMHD1 is its phosphorylation which can take place in many residues. Even though several sites have been identified where SAMHD1 can be phosphorylated on, the one of greatest importance is T592. There is a shift in phosphorylation status of SAMHD1 protein between cycling and non-cycling cells. When this residue is phosphorylated, SAMHD1 appears to lose its ability to inhibit retroviral infections, nonetheless this seems to be an opposing mechanism to nuclear localization as T592 phosphorylated SAMHD1 appears to translocate in the cytoplasm. However, dNTPase capacity, oligomerisation, and binding to dsRNA abilities, remain unaffected[133-136]. By using a phosphomimetic mutation at residue 592 (T592E), SAMHD1 tetramerization stability was weakened, alongside with a decrease in dNTPase efficacy. This feature was based on conformational changes in the structure of the tetramer driven by the mutated residue. Whether this important observation applies also to the normally phosphorylated threonine residue, or if this specific phosphorylation changes drastically the structure interface is yet unclear[102,137].This phosphomimetic SAMHD1 protein was tested also in PC3 cells (a prostate cancer cell line) and influenced drastically the cell cycle as cells accumulated in the transition between S and G_2 and cell proliferation was inhibited[136]. T592 phosphorylation appears to modulate not only activity of hydrolysing intracellular dNTP pools but also for regulating DNA replication and cell proliferation since it promotes normal fork progression[107].

In CD4+ T cells, treatment with dasatinib, a tyrosine kinase inhibitor (TKI) can prevent SAMHD1 phosphorylation and thwart HIV-1 infection, presenting once more the importance of SAMHD1 against viral infections. The exact same result was described in peripheral blood mononuclear cells (PBMCs) retrieved from chronic myeloid leukaemia (CML) patients after treatment with several TKIs that are used in CML treatment regimens and dasatinib being one of them. PBMCs obtained from CLL patients that had been

treated with dasatinib for longer than 2 years, confirmed lower phosphorylation levels in comparison to untreated controls[138-140].

A group of interleukins, namely IL-2, IL-7 and IL-15 can induce SAMHD1 phosphorylation in CD4+ T cells, without altering total SAMHD1 levels, for at least 48 hours post treatment[139,141]. Increase of IL-7 mediated SAMHD1 phosphorylation in CD4+ cells was further confirmed *in vivo*[139]. Regulation of IL-7 and IL-15 is under the JAK pathway. Treating CD4+ cells with two JAK inhibitors, Ruxolitinib and Tofacitinib, abolishes SAMHD1 phosphorylation, but only when it is IL-15 induced, which stresses even more the alternate paths to SAMHD1 phosphorylation[141].

Another residue that can be phosphorylated is S33 and it can be phosphorylated both in proliferating and quiescent cells. Phosphorylated S33 has only been shown to be required for inhibition of retrotransposition of the long-interspersed element 1 (LINE-1), but for none other of the main SAMHD1 functions including cellular localization, HIV-1 inhibition, dNTPase activity or oligomerisation[142]. Interestingly though, inhibition of endogenous retroelements can also happen by T592 phosphorylation and direct binding at the ORF2 protein of the like LINE-1 elements (L1)[143]. Moreover, SAMHD1 can interact with LINE-1 also in a non-phosphorylated dependent manner. As mentioned earlier, even though SAMHD1 contains a localisation signal and through that it is located at the nucleus, for some of its abilities it needs to be shuttled to the cytoplasm. So, in order to suppress LINE-1 retrotransposition, it is transferred via exportins to the cytoplasm[19]. A total of 7 SAMHD1 mutations have been found in CLL and/or colon cancer (CC) and they appear to have limited ability towards inhibiting LINE-1 retrotransposition. However, as described earlier, even though the enzyme's hydrolytic ability is not related and does not affect the capacity to inhibit LINE-1 retroelements, the ability to bind and regulate ORF2, appears to be crucial for LINE-1 inhibition[144].

2.12.2 SAMHD1 dephosphorylation

SAMHD1 dephosphorylation at the T592 residue occurs in a cell cycle dependent manner and more specifically during the mitotic exit in the M/G_1 transition[145,146]. With the use of HEK293T cells as an *in vitro* model, the responsible phosphatase for this was serine / threonine protein phosphatase 2A (PP2A) which binds to the regulatory unit B55α and forms a holoenzyme which in turn removes the phosphatase from residue T592. This activity has been described both in cycling (HeLa and CD4+) and non-cycling (MDM) cells[146]. However, contradicting results from a different study named PP1 as the main SAMHD1 phosphatase instead, arguing that probably more than one phosphatases are involved in this process[145].

2.12.3 Cell cycle regulation of SAMHD1

SAMHD1 has been shown to be highly expressed during G_1 phase of the cell cycle and is detected at its lowest levels during S phase. Knocking down SAMHD1 in skin and lung fibroblasts, resulted in cell cycle progress disruption, as the dNTP pools expanded to such an extent, that cells accumulated in G_1 phase, and cell growth occurred at slower pace[25]. An early study that used HeLa cells as a model and mass spectrometry (MS) as a readout, recognized SAMHD1 as a substrate for cyclin dependent kinase 1 (CDK1). A finding that was later confirmed as it was discovered that SAMHD1 is phosphorylated by CDK1 kinase at the recognition motif ^{592}TPQK^{595}, and more specifically at residue T592[133,147]. Through MS, it was identified that SAMHD1 was associated with cyclin A2/CDK1 only in proliferating cells, in a cell cycle dependent manner. SAMHD1 was practically not detected in G_1/S phase and was steadily increased by late S phase. It was also shown that it forms complexes with cyclin A, and treatment with etoposide elevated the interactions between cyclin A and SAMHD1 and that was followed by an induction in T592 phosphorylation[24,103,134].

Even though initial studies presented CDK1 to be the sole phosphorylating kinase of SAMHD1 after interaction with cyclin A, and not the complexes of cyclin A / cyclin dependent kinase 2 (CDK2) or cyclin E / cyclin dependent kinase 3 (CDK3)[103], it later became more apparent that more cell cycle regulated molecules could interact with SAMHD1, including CDK2 and S-phase kinase-associated protein 2 (SKP2). Nevertheless, CDK1/2 with cyclin A2 are the main kinases phosphorylating SAMHD1 at the T592 residue. Interestingly, many of these kinases exhibited distinct functional and expression patterns, which could implicate that more than one kinase can phosphorylate SAMHD1, but these different phosphorylations might be dependent on the cell cycle stage and/or the cell type. CDK2 presented constant expression levels in cycling and non-cycling U937, as well as THP-1 cell lines, even though in HIV-restricting non-cycling cells, proteins that interact with SAMHD1 phosphorylation are usually downregulated. Also, SKP2 despite being bound to SAMHD1, did not initiate phosphorylation at the T592 residue[148].

Cyclins A2 and E bind directly to residues located at the C-terminus of SAMHD1 for enabling CDK2 phosphorylation and as mentioned earlier the C-terminal of the protein is imperative for the formation of the homotetramer. Jang et al, showed that cyclin A2 can bind to SAMHD1, irrespectively of its tertiary form, with a preference however to monomers and dimers, in order to induce homotetramer dissociation and inhibit its hydrolytic activity[92,149].

Opposed to the study performed in non-transformed lung and skin fibroblasts that described altering levels of SAMHD1 protein expression during cell cycle, another study on monocytes and T-lymphocytes, showed that SAMHD1 expression remains stable throughout the entirety of the cell cycle but it is the phosphorylation levels that fluctuate

depending on the cell cycle phase. These contradictory results could be cell specific and depend on the expression levels of SAMHD1 in each cell line or correlate with different SAMHD1 regulatory mechanisms in different cell types. Another reason could be different experimental approaches, as changing the variables of an experiment could affect the outcome.

Phosphorylation at the T592 residue was entirely abolished during G_1 phase, which was in accordance with the change of cyclin A2 levels through the cell cycle, pointing out the role of cyclin A2 in SAMHD1 phosphorylation[149]. SAMHD1 phosphorylation is initiated prior to the beginning of S-phase by cyclin E/CDK2 complex and is therefore maintained through G_2 phase until mitosis by cyclin A2/CDK2 complex, illustrating the complexity and strict regulation that SAMHD1 phosphorylation is under[145]. Finally, although T592 phosphorylation can influence the intensity of the dNTPase activity, the hydrolytic activity of the enzyme remains functional irrespectively of the phosphorylation status[145,149].

The family of cyclins involved in SAMHD1 phosphorylation and cell cycle regulation, was extended by two more cyclins as downregulation of cyclin E2, a specific CDK2 binding partner, as well as cyclin D3 which can also bind at CDK2, decreased HIV-1 infection levels in MDM cells[150]. Inhibition of cyclin D3, led to reduced CDK2 activation and decreased T592 phosphorylation levels of SAMHD1[150]. T592 phosphorylation by the cyclin E2/CDK2 complex also plays a more important role against hepatitis B virus (HBV) in HCC, compared to other cyclins, as co-immunoprecipitation (Co-IP) experiments showed a direct binding between SAMHD1 and CDK2[151].

Different studies including either the use of small interference RNA (siRNA) or CDK inhibitors, in HIV-1 infected MDM cells, verified that kinases CDK2 and CDK6, could simultaneously regulate cell cycle and phosphorylate SAMHD1. Something that could not be verified for kinases CDK1, CDK4 and CDK5[152]. Furthermore, CDK2 is downstream of CDK6 and that it could be either directly or indirectly regulated by CDK6[152,153]. To support this, in MDM cells infected with either HIV-1 or Herpes Simplex Virus type 1 (HSV-1), treatment with Palbociclib, a specific CDK4/6 inhibitor in a dose dependent manner, remarkably decreased CDK2 phosphorylation levels but not CDK6 expression levels and reduced intracellular dNTP pool levels[153,154].

In MDMs expression of p21, also known as cyclin dependent kinase inhibitor 1A (CDKN1A), inhibits CDK1 expression which consequently results in reduced SAMHD1 phosphorylation levels[155]. A direct correlation between reduced levels of SAMHD1 phosphorylation and p21 was also observed in MDDCs, where p21 is highly expressed[156]. In granulocyte - macrophage colony-stimulating factor (GM-CSF) differentiated MDMs, where cells present a non-proliferating phenotype, cyclin D2, which is G_1/S specific, can restrict HIV-1 infection through a SAMHD1-dependent manner. In this specific cell type, a complex between Cyclin D2, CDK4 and p21 is formed, which blocks CDK4 from phosphorylating

SAMHD1[157]. SAMHD1 has a cyclin – CDK recognizing motif (RXL motif) that cyclin A2/CDK complex can bind to. This binding sequence has also been shown to be very important for the enzyme's stability, tetramerization and hydrolase activity[112,158].

Treatment with two different topoisomerase inhibitors, etoposide and camptothecin that induce DNA damage, led MDM cells into cell cycle arrest and the cells' return to G_0 phase. Even though total SAMHD1 levels were not modified, a decrease in T592 phosphorylation occurred. This can be explained because these inhibitors upregulate p53, which in turn upregulates p21 that finally decreases phospho-SAMHD1 levels[159]. These results were supported with the use of another topoisomerase inhibitor, neocarzinostatin (NCS). It should be noted however that in the case of NCS-induced inhibition, SAMHD1 dephosphorylation was DSB-specific and did not occur when DNA was damaged as a result from UV irradiation[160].

2.13 AML and T-ALL; Can we improve treatment, or have we reached a plateau?

2.13.1 Acute Myeloid Leukaemia

Acute Myeloid Leukaemia (AML) is a haematological malignancy characterized by the persistent proliferation of myeloid cells. These immature and poorly differentiated cells clonally expand in the bone marrow (BM), leading to BM insufficiency. The median age of diagnosis is approximately 70 years. Even though overall prognosis is poor, this fact is highly dependent on age, as prognosis deteriorates the older the patients are[161]. Paediatric AML consists of around one quarter of all recorded paediatric leukaemias[162,163]. Nonetheless, overall survival rates in paediatric AML have improved within the last decades to around 80%, mainly due to treatment intensification and the application of minimal residue disease (MRD) to stratify for haematopoietic stem cell transplantation[1,164]. Intensification of treatment may be a favourable solution for younger patients but on the other hand, it is a major drawback when treating older patients. Many of these patients do not tolerate intensive chemotherapy, so intensifying treatment is not applicable and even though many studies have been performed targeting these unfit for treatment patients, results remain unsatisfactory[165].

Current treatment protocols for AML induction therapy treatment usually combine anthracycline with cytarabine. In the so-called 7+3 protocol, the numbers refer to the total amount of days each compound is given to the patients. Anthracyclines are administered for 3 days with the one included in the protocols most often being daunorubicin (but idarubicin or mitoxantrone can also sometimes be included), while cytarabine is given for 7 consecutive days. Cytarabine is a NA, and its active metabolite cytarabine triphosphate (ara-CTP) has a similar incorporation rate at the elongating DNA strands as dCTP. Ara-CTP incorporation leads to DNA extension pause at the replication fork and therefore cell

death[166]. Consolidation therapy does not follow a universal protocol, but it usually includes high doses of cytarabine (HDAC)[167,168].

Protocols haven't altered significantly for many years, and the modifications and adjustments have only supplemented the ongoing standard treatment in the presence of a detected mutation. One such example is the addition of midostaurin, a kinase inhibitor, to treat patients with *FLT3* (FMS-like tyrosine kinase 3) mutation[169]. Furthermore, since AML can have many genetic aberrations, due to the newest advances in sequencing techniques these genetic mutations can be timely diagnosed, and more precise and effective therapeutic approach can be given to patients[170].

2.13.2 T-cell lymphoblastic leukaemia

Acute lymphoblastic leukaemia (ALL) can be divided in two distinct categories, T-cell and B-cell based on the affected lineage and both are characterized by rapid proliferation of immature lymphocytes and subsequent overpopulation by these ineffective cells in lymphoid organs and the BM. The applied treatment regimens are similar in both entities, nonetheless, the response and outcome can be different.

Paediatric T-cell acute lymphoblastic leukaemia (T-ALL) represents around 15% of all diagnosed ALL cases and in the adult population they are higher as they consist of 25% of all ALL cases[171,172]. Older patients have worse prognosis. Although many protocols that were initially used for paediatric ALL treatment have been used as a scaffold and developed for adult treatment regimens, overall prognosis has not improved to the levels of Paediatric ALL [173]. Even though event-free survival (EFS) has improved to over 85% in children, intensive chemotherapy is mostly required to reach it, and as in the case of AML, older patients might not tolerate intensive therapy[171]. Finally, even though it can be classified as an entity with high cure rates, prognosis for relapsed T-ALL patients is dismal, with only 1 out of 10 patients reaching 5-year survival[174].

Many different approaches have been introduced in treatment of T-ALL and relapsed / refractory T-ALL (R/R T-ALL) including chemotherapy, antibody targeted therapies and chimeric antigen receptor (CAR) T-cell therapy, with the latter one being still experimental and in early clinical trials[174]. One of the chemotherapeutic compounds that showed promising results in both paediatric and adult T-ALL was a NA named nelarabine and was explicitly toxic against T-cells even at low concentrations[175]. A very promising result, during the first phase I clinical trial where 93 patients (34 of which were children) were treated with nelarabine was reported. In patients suffering from T-cell malignancies, more than half achieved partial or complete remission even though most of them had already experienced at least two relapses. However, even as promising as these results could be, severe neurotoxicity events were reported in high and moderate doses, which could limit drug application. Patients were administered the drug in a range of 7 different doses, each treatment cycle was 5 days and the median number of cycles for the treatment per

patient was one. Higher doses of nelarabine were associated with neurotoxicity, but half of the children and 85% of the adults presented reversible neurotoxicity[176]. A systematic review later showed that use of nelarabine as a first line treatment of R/R T-ALL patients was effective and increased the percentage of patients that reach CR. It also showed that combining nelarabine with ongoing chemotherapy can increase survival and MRD levels were undetectable[177]. Eventually, a large phase 3 trial incorporating nelarabine in front-line T-ALL treatment has shown moderate survival benefits for patients treated with nelarabine[178].

2.14 SAMHD1 expression, mutations, and deregulation in various cancer types

2.14.1 Impacts of mutated SAMHD1

Many somatic mutations have been reported in SAMHD1 and they are frequently observed in cancer cases, as they are presented at the COSMIC (catalogue of somatic mutations in cancer) database. So, it is of no surprise that in many of the sequencing studies that have been performed, followed up by functional experiments, presence of mutated *SAMHD1* could have a direct effect in pathophysiology of the disease, and its treatment thereof. In a study where primary skin fibroblasts acquired from AGS patients with inactivating mutations of *SAMHD1*, dNTP pools were imbalanced to a high extent and the levels of all respective dNTPs were unevenly enlarged, with purine dNTPs demonstrating the biggest difference. This promoted a mutagenic state with increased rates of mutations in the genome, naming SAMHD1 as a caretaker of the genome. This finding led to the assumption that the physiological function of SAMHD1 is of extreme importance regarding cell homeostasis and that the protein operates as a tumour suppressor. Nonetheless, SAMHD1 deficient fibroblasts could eventually adapt to these unbalanced intracellular pools and proliferate at a normal rate *in vitro*, however, with probable accumulation of somatic mutations[179]. In many cancer types, mutations of *SAMHD1* have been reported and the most common effect presented is decrease in protein levels and deregulation of the dNTPase activity, which in turn elevates dNTP levels intracellularly.

One interesting mutation is the K366C/H, that has been described in leukaemia and colon cancer cases but not AGS. It is structurally stable and can form homotetramers, total protein levels are not decreased, but remarkably displays loss of the dNTPase activity probably due to conformational changes in the tertiary structure. This finding highlights the importance of sequencing samples from patients and not just rely on tissue staining, if SAMHD1 is to be used as a biomarker for clinical diagnosis in different cancer types[180].

2.14.2 Mutations in haematological malignancies

PBMCs collected from a group of 8 patients with SS, a subtype of CTCL, showed 3 times lower *SAMHD1* mRNA levels compared to healthy donors and the promoter of *SAMHD1* was methylated at a 51-fold rate more compared to healthy individuals[181]. Also, a case report presented one patient who suffered from AGS and had a homozygous mutation of *SAMHD1* that later exhibited an aggressive type of epidermotropic CTCL[182]. In CD4+ cells derived from 15 SS patients, SAMHD1 protein expression levels were always lower compared to healthy donors, however, there was no variance in *SAMHD1* transcript expression, which suggests the protein level difference was based on translational regulation. Furthermore, miR-181 levels were elevated compared to healthy donors and in all patients, and as expected there was an inverse correlation between miR-181 and SAMHD1 expression levels. These findings were independent of disease progression or current ongoing treatment[124].

Analysis of PBMCs from 22 AML patients, showed a differential expression pattern among all the samples. Based on the established levels from a known cell line as a comparison, levels on the PBMCs varied from no expression to very high expression. It is of great interest that the expression levels of SAMHD1 are inversely correlated with the myeloblast percentage in the isolated patient PBMCs[79]. Furthermore, in normal BM, SAMHD1 was not expressed while in AML blasts it was present, thus establishing SAMHD1 expression as a candidate for being a biomarker of malignancy[183]. Contrary to this finding, in a small group of 16 AML patients, cells derived from the BM displayed lower SAMHD1 levels compared to a non-AML group, which could be an indication that SAMHD1 expression can be assisting in suppressing AML development[184]. However, this study showed no correlation among SAMHD1 expression and either apoptotic or autophagic markers was reported, even though for more solid results, a bigger cohort study should also be evaluated[184].

In a cohort including 361 patients, carriers of the G allele in the SAMHD1 single nucleotide polymorphism (SNP) rs6102991 showed decreased risk of non - complete remission (CR) compared to the carriers of the A allele, after ara-C based induction therapy[185]. A bigger study that included three paediatric cohorts with children diagnosed with *de novo* AML, identified 3 more SNPs in the 3´-region of *SAMHD1* to be significantly associated with the clinical outcome. The most interesting finding concerned SNP rs7265241, where presence of variant G allele, exhibited worse EFS and overall survival (OS) in all three cohorts[186].

From samples collected from a cohort of 108 paediatric leukaemia patients and comprised of AML, B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) and T-ALL, a positive correlation between FLT3 kinase and SAMHD1 was established. FLT3 is deregulated in many cancer forms and two different cell lines expressing mutated FLT3 showed a decrease in SAMHD1 phosphorylation. A finding that could not be replicated in

a cell line carrying a non-mutated *FLT3*, which highlights the connection between SAMHD1 and FLT3 only in pathological conditions[187].

In a cohort of 53 relapsed CLL patients, *SAMHD1* mutations were shown to have a detrimental role. These mutations were relapse gene drivers, they negatively affected treatment outcomes and they induced resistance in chemotherapy. Sub-clones of cells that harbour these mutations appear to be enriched after treatment, highlighting their contribution towards a negative outcome[188]. In R/R CLL, *SAMHD1* mutations appear at a higher incidence. In a cohort of 92 patients with R/R CLL, 11 of them (10%) presented *SAMHD1* mutations suggesting that resistant clones are more probable to carry *SAMHD1* mutations, and in 6 out of these patients, more mutated genes were also reported[189]. Germ line *SAMHD1* mutations can also predispose for CLL. In *SAMHD1* mutated CLL cells, significantly lower levels of *SAMHD1* mRNA expression levels were detected and through sanger sequencing, it was discovered that there were patients that their transcripts were entirely comprised of mutated *SAMHD1*, as well as that patients with R/R CLL have a 4-fold higher frequency in *SAMHD1* mutation occurence[24]. Furthermore, in the same study it was displayed that mRNA levels and protein levels among CLL are heterogeneous and there are more regulatory mechanisms, since non-mutated *SAMHD1* CLL also showed diminished SAMHD1 protein expression[24].

T-cell prolymphocytic leukaemia (T-PLL) is a very aggressive type of leukaemia. In a cohort comprised of 33 patients, *SAMHD1* was found to be either mutated or have copy number variations in 24% of the cases. It was the second most frequently mutated gene regarding genes involved in DNA repairing, and most mutations were either frameshift or nonsense. These mutations affected both mRNA and protein expression levels and compared to T-PLL samples with wild type *SAMHD1*, dNTP levels were significantly elevated[190].

Recently, several studies have reported *SAMHD1* mutations in mantle cell lymphoma (MCL), an aggressive type of B cell lymphoma with poor prognosis. A study of 82 patients identified *SAMHD1* for the first time as a driver gene mutation. It was either mutated or deleted and these alterations were present in 10% of the cases but limited only in one of the two MCL subtypes[191]. One small study described four missense mutations for the first time. Three patients in that cohort shared the same R451H, interestingly though, the protein expression patterns were different in all of them. Whether this was due to additional mutations or because of other regulating proteins / mechanisms, is to be explored[192]. A larger study that involved 182 patients from two trials, identified that 7.1% of the patients carried at least one mutation and no correlation between SAMHD1 and failure free survival (FFS) or CR was identified. Biopsy samples from the patients, showed that SAMHD1 expression was mildly correlated with cell proliferation[193]. In a smaller cohort, *SAMHD1* mutations were found in 8.5% of the patients, and most of them carried a mutation in another gene as well. Even though this cohort was small to predict any

potential association between treatment and survival rates, it did demonstrate *in vitro*, that patients carrying mutations in *SAMHD1* developed resistance to either cytarabine or fludarabine treatment, two NAs that are currently used for treatment[194].

In a larger study, SAMHD1 protein expression varied to wide range (with median value of 69% of the cells being stained positive) and there was a direct correlation between mRNA and protein levels, in contrast to the results derived from the CLL studies. Additionally, SAMHD1 was differentially expressed based on cell morphology, which is a standard classification method for MCL, with cells not having classical morphology (comprising around 10% of all cells), expressing higher levels of SAMHD1[195]. Another MCL study confirmed that almost two thirds of the patients show SAMHD1 expression, 20.7% out of which exhibited strong positive signal[192].

Hodgkin lymphoma (HL) is another type of haematological malignancy in which the potential role of SAMHD1 as a prognostic marker has been assessed. Lymph node biopsies were assessed from patients prior to treatment and in more than 30% of them, the characteristic Hodgkin and Reed Stenberg cells (HRS) were positive for SAMHD1. This expression was nevertheless unrelated to other clinical and pathological factors of the disease and protein expression in patients treated with standard of care regimens for classical HL associated significantly with disease specific survival (DSS) and OS[196].

2.14.3 SAMHD1 mutations in solid tumors

In a panel of 5 different solid tumours primary cells were retrieved and SAMHD1 expression was assessed, with variable results depending on the cancer type being extracted, showing that there cannot be a universal approach for targeting SAMHD1 in all cancer types. In rectal carcinoma (RC) SAMHD1 levels were high in all patients while in pancreatic cancer the phenotype was the complete opposite with very low levels in all tested samples. Generally, SAMHD1 expression was linked to poor differentiated histology and high grade. In breast cancer (BC), OC and NSCLC, SAMHD1 acted as a negative prognostic marker[197]. Samples derived from GC patients showed that SAMHD1 is downregulated in comparison to the adjacent physiological tissues. Furthermore, protein expression levels were directly associated with the size and the stage of the tumour as well as with invasion depth[131].

In a small cohort of 34 patients with BC, half of the cases presented either lower levels of SAMHD1 protein or it was complete absent[24]. In one GC patient, one mutation that is present in the C-terminus of SAMHD1 (K484T), does not affect the hydrolytic activity of SAMHD1 but instead has a direct effect in promoting a DNA repair mechanism. Because of this mutation, the binding competence to other nucleases is disabled, thus genome integrity is compromised[104]. Furthermore, in OC patients, high levels of SAMHD1 linked with high grade serous histology. Nevertheless, all patients with mutated *BRCA1/2* genes showed high *SAMHD1* expression, but there was no significant association among these

genes. Finally, higher levels of *SAMHD1* correlated with worse OS and progress free survival (PFS)[129].

In colorectal carcinoma (CRC), *SAMHD1* has been identified as one of the most mutated genes involved in dNTP metabolism. Mutations appear non-randomly in functionally significant residues that play an integral part in the dNTPase activity, and this leads to imbalanced dNTP pools and elevated mutation rates. Deletion of one allele in CRC-related mutations in mouse embryos, presented a similar effect[198]. As with other cancer types expression of *SAMHD1* is lower in CRC patients compared to healthy donors. Higher expression levels are correlated with poor prognosis, higher cell proliferation rates and significantly higher probability for metastasis[199,200]. More specifically, regarding stage II colon cancer, SAMHD1 can be treated as a prediction marker since low expression of SAMHD1 was associated with a better 5-year OS compared to the group expressing high levels of SAMHD1. Unrelatedly to the stage though, adjuvant chemotherapy appears to be beneficial for patients with low SAMHD1 levels. However, due to the study design that reported this, it was not possible to extract more focused data whether SAMHD1 could have a predictive role against specific drugs as there was limited information about which treatment was followed for each patient. Furthermore, because this was a retrospective study, validation of SAMHD1 via immunohistochemistry (IHC) was difficult to be performed for all the patients[201]. Finally, SAMHD1 expression has been shown to be negatively correlated with Kirsten rat sarcoma viral oncogene homologue (KRAS), a gene that is mutated in almost half of the CRC cases[200].

High *SAMHD1* expression is correlated with poor prognosis in LAC[130]. In a small cohort of 5 patients diagnosed with LAC, tissue collected from the cancer site showed significant downregulation both in mRNA and protein levels compared to healthy adjacent tissue. SAMHD1 promoter methylation was detected in the pathological tissue, explaining the low levels of SAMHD1[202]. Followed up by a larger study of over 200 LAC patients, mRNA levels were confirmed to be lower compared to the neighbouring healthy tissue and this trait was related to advanced disease[111]. Hypermethylation of *SAMHD1* promoter and lower mRNA levels were also considered as a marker linked to poor prognosis in skin cutaneous melanoma (SKCM)[203].

Tumour samples collected from glioblastoma patients, illustrated a significantly higher SAMHD1 expression compared to normal samples. However, the main reason that treatment outcome was affected is not based on the dNTPase activity of the enzyme, but rather on its assistance towards HR. SAMHD1 presence induces cell proliferation both *in vitro* and *in vivo*. Vpx-mediated SAMHD1 depletion sensitized cells to PARP inhibitors, thus inhibiting the DSB repair function that SAMHD1 confers to the cells. In addition, SAMHD1 depletion improved irradiation therapy which induces DNA DSB[204].

2.15 Nucleoside analogues in cancer treatment

2.15.1 Role and activation of nucleoside analogues

Targeting nucleotide metabolism to inhibit cell proliferation, is a common goal in cancer treatment. One vast and very diverse category of compounds that has been used among the discovery of the first chemotherapeutic regiments are the NAs, a subclass of antimetabolites, as they have been used for treatment in both haematological malignancies and solid tumours. NAs share common characteristics and mimic physiological nucleosides; however, they have minor chemical alterations either at the sugar or at the base moiety (**figure 4**). Their main course of action is either to incorporate into the elongating DNA or RNA molecules or inhibit different enzymatic activities. Furthermore, some of them have been shown to be tissue specific, and their cytotoxicity could also differ among different pathological conditions[166,205].

Several studies have demonstrated that one reason behind this discrepancy could be the nucleoside transporters (NTs). There are two main transporter families: the equilibrative nucleoside transporters (ENTs) and the concentrative nucleoside transporters (CNTs). Their role is to import nucleosides (and their analogues) into the cell. Cytotoxic effects of the NAs can vary due to the differential tissue localization of the NTs as well as the variance among them in specificity and selectivity based on different chemical group uptake[206-209].

2.15.2 SAMHD1 efficacy against nucleoside analogues

Since it was interesting to know whether NAs could interact with SAMHD1, sensitive techniques were required to be able to verify if NA-TPs could be bound to SAMHD1 either as allosteric activators or as substrates. These developed coupled enzyme techniques either used inorganic pyrophosphatase (PPase) from *E. coli* or exopoly-phosphatase Ppx1 from *S. cerevisiae*. Both essays used as a template PPPi which is the product after the dNTP hydrolysis from SAMHD1. Both techniques have their respective advantages. PPase gives stronger signal since it cleaves PPPi into 3x inorganic monophosphate (Pi) and demands less time to purify the enzyme for the assay. On the other hand, since Ppx1 cleaves PPPi into pyrophosphate (PPi) and Pi, not only can it be used for an endpoint assay, but it can also be used as a continuous assay to monitor quantitative kinetic parameters[210,211]. Later, another technique based on enzymatic activities was described, where SAMHD1 phosphorylase activity was coupled with the pyrophosphatase from *E. coli* as described before and included malachite green as a readout. With this high-throughput technique compounds activating or inhibiting SAMHD1 or compounds that could act as substrates could be identified[212].

One of the first studies where the question whether SAMHD1 can affect HIV-1 NA treatment via its triphosphohydrolytic activity towards nucleoside reverse transcriptase

inhibitors (NTRIs), was performed by Amie et al. NRTIs are used against HIV-1 infection, and they lack the 3´-OH group at the ribose. Absence of this hydroxyl group was however a restrictive factor that prevented NRTI-TPs from SAMHD1 hydrolysis. [76]. The same group also presented evidence for the first time that NA-TPs with different base modifications could be hydrolysed by SAMHD1, including the non-canonical deoxyuridine triphosphate (dUTP)[72], followed by a study of Ballana et al, where they discovered that depending on the presence or absence of SAMHD1 in MDMs and PBMCs, NTRI thymidine analogues had a different outcome in viral infection restriction. When SAMHD1 was depleted, reduced viral sensitivity to the analogues was observed probably due to an increase of intracellular dNTPs that resulted in competition with the thymidine analogues[213]. Moreover, the triphosphates of two dG analogues called acyclovir and ganciclovir and are used against different viral infections demonstrated different results. Via an enzymatic assay, acyclovir was recognised as an allosteric activator, but ganciclovir was not, and both of them were refractory to SAMHD1 hydrolysis. On top of that, only at the presence of acyclovir-TP could other dNTPs be hydrolysed by SAMHD1 but not ganciclovir-TP. This inconsistency in the outcome of hydrolysis, clearly depicted the tight interactions among different sites of the enzyme with the different NAs, and in this case only the presence or absence of one hydroxyl group can drastically affect the enzyme's catalytic activity[210].

Depleting SAMHD1 expression with Vpx treatment in differentiated THP-1 myeloid cells, altered HIV-1 infection rates, indicating that SAMHD1 expression and use of NAs can potentially alter the treatment outcome[214]. But the limiting factor towards SAMHD1 dNTPase activity against NAs is that not all binding pockets allow incorporation of the same NAs. AS2 is more restricting compared to CS which allows a greater group of NAs to be hydrolysed[215]. Some NAs can act as substrates only for the CS and some for both AS2 and CS. Small alterations between dNTPs and their analogues do not influence CS binding since they cannot cause architectural changes at the enzyme, and they form similar conformations as per the canonical dNTPs inside the pocket. Even though they can act as substrates, one big discrepancy that can rise though depending on the modification is that they can have significantly lower hydrolysis rates compared to the canonical dNTPs, which could act beneficially for treatment as the NA-TPs would occupy SAMHD1 CS longer and allow the remaining active metabolites act in a cytotoxic manner. On the other hand, only minor modifications in the 2´-sugar moiety are permissive for NA binding at AS2, even though these (or even bigger) modifications are not restrictive regarding CS binding[215].

2.15.3 Known compounds as SAMHD1 substrates

There have been several studies *in vitro* that examined different NA compounds relevant to cancer treatment and for many of them, using THP-1 cells as a model, SAMHD1 depletion resulted in treatment sensitization to a greater or a lesser degree[216]. These observations can only prove the significance of the difference between NA-TPs and dNTPs as well as the specificity of each compound in the different cell systems where they are tested. On the other hand, it should be noted that not all active metabolites are affected by SAMHD1 as no change at the active metabolites of gemcitabine or 6-thioguanine was reported, which is directly related on the incapacity of SAMHD1 to accept their modifications in their base or sugar ring compared to the physiological dNTPs[80,217].

One of the first compounds to be confirmed as a substrate for SAMHD1 was a compound used against leukaemias named clofarabine. Results showed that clofarabine could be

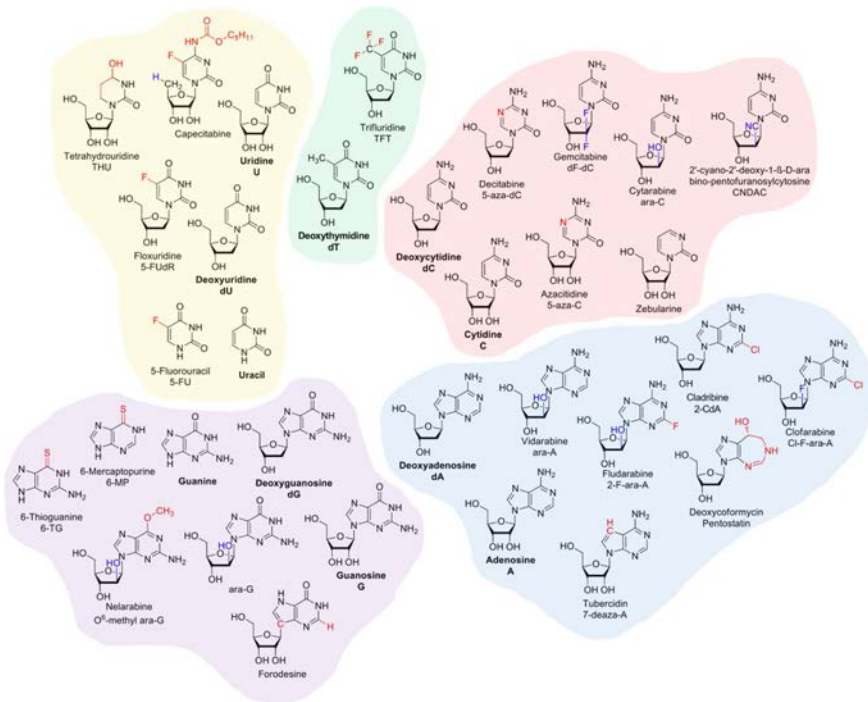

Figure 4. Schematic representation of different categories of nucleoside analogues that can be used for treatment. Modifications in sugar moiety are presented in blue and base modifications are presented in red.

(Source: Tsesmetzis N et al, **Nucleobase and Nucleoside Analogues: Resistance and Re-Sensitisation at the Level of Pharmacokinetics, Pharmacodynamics and Metabolism,** *Cancers (Basel) 2018 Jul 23;10(7):240. doi: 10.3390/cancers10070240)*

hydrolysed at rates like those of canonical dNTPs[210] and not only could clofarabine be used as a substrate but also as an activator at AS2. In a panel of 133 hematologic and lymphoid cells lines a significant correlation between low SAMHD1 expression and clofarabine cytotoxicity was revealed, showing the negative role of SAMHD1 towards treatment[80,216]. Ara-C, a cytosine analogue that has been used as a golden standard in AML therapy, can be used by SAMHD1 as a substrate but not as an activator, and its hydrolysis rate is less efficient compared to dCTP[80]. Fludarabine is another example of NAs that can be hydrolysed, but still not as efficient as ara-C. Even though SAMHD1 is the main protein affecting the treatment result, interactions with other proteins should be considered as they can also indirectly alternate the outcome. One such example is NONO, where it stabilizes SAMHD1 and increases its half-life, thus rendering AML cells resistant to ara-C treatment[67].

A great example of SAMHD1 use as a prediction marker for treatment regimens when using drugs that serve the same purpose is depicted with decitabine (DAC) and 5-azacytidine (5-aza). Both compounds are hypomethylating agents belonging in the family of cytidine analogues and are used in treatment against myelodysplastic syndrome (MDS) and AML. They share similarities with each and the only difference between them is an additional 2´-hydroxyl group in the 5-aza ribose compared to the deoxyribose sugar of DAC. This distinction amongst these two compounds, renders DAC to act both as an allosteric activator for AS2 and a substrate for CS, while 5-aza cannot bind at any SAMHD1 site. BM samples from patients treated with either of the two compounds, confirmed an inverse correlation between SAMHD1 protein levels and DAC treatment, but not for 5-aza. Furthermore, xenotransplanted immunodeficient mice with AML cell lines, had prolonged survival when injected with SAMHD1 depleted cells followed by DAC treatment, compared to the parental cell line. As per with the *ex vivo* samples, no SAMHD1-dependent effect was shown in 5-aza treated mice[218]. This correlation between the predictive significance that SAMHD1 concurs can also be extended to solid tumours. In OC and NSCLC, increased levels of SAMHD1 are related to poor response to NA and platinum-based therapies. A correlation not found in BC treatment regimens[197].

In murine bone marrow derived macrophages (mBMDMs), SAMHD1-deficient cells were more sensitized to the combination treatment of forodesine and dG, compared to single drug treatment where no effect was displayed[97]. Forodesine is a highly specific inhibitor of purine nucleoside phosphorylase (PNP), that exhibits high specificity against T-cell derived malignancies in the presence of dG. PNP is the enzyme that under normal conditions degrades dG, so PNP inhibition leads to dG accumulation, which in turn is phosphorylated by dCK to dGTP. These escalating dGTP intracellular levels, result in dNTP pool imbalance-induced cell death[219,220]. This effect of synergistic treatment between forodesine and dG was extended also in PBMCs derived from CLL patients. Cells that

carried *SAMHD1* mutations, were significantly more sensitized to treatment compared to non-mutated *SAMHD1* cells[97].

Nelarabine is another example of SAMHD1 interference towards treatment outcome. It is the water-soluble prodrug of ara-G, and it has been shown to be explicitly cytotoxic against T-ALL but not against B-ALL. Nelarabine is converted to ara-G through demethoxylation by adenosine deaminase (ADA)[221]. After comparing multiple T-ALL and B-ALL cell lines, as well as patient samples collected from both malignancies, from various datasets, *SAMHD1* levels in T-ALL cells were significantly lower reasoning to an explanation of this inconsistency in nelarabine treatment. In protein level, almost all T-ALL cell lines were negative for SAMHD1. Despite having higher protein levels, when B-ALL cells were treated with Vpx, they responded better to nelarabine. Finally, as stated earlier, *SAMHD1* promoter methylation downregulates protein expression, and in T-ALL *SAMHD1* gene promoters were highly methylated, supporting the hypothesis of SAMHD1-controlled responses to nelarabine treatment[222].

In a group that consisted of 15 different cell lines from 3 different B-cell malignancies, SAMHD1 expression levels were evaluated, showing a wide range across the panel. Different drugs used for MCL treatment were tested, and out of them, cytarabine was the sole compound to illustrate correlation between SAMHD1 expression and treatment sensitivity. A correlation that was supported by another study as well[192]. However, none of the other compounds showed any association, including fludarabine, a purine NA, that is known to be hydrolysed by SAMHD1, showing once more that SAMHD1 activity is cell-type dependent and its effectiveness relies on the expression of other enzymes[193,216].

Finally, in a human colon cancer cell line that expressing SAMHD1 protein, cells that carried the D137N mutation that leads to SAMHD1 dNTPase deficiency, showed decreased levels of apoptotic markers, thus showcasing the importance of functional SAMHD1 in halting cancer development. On top of that, treatment with 5´-fluorouracil (5-FU), a NA used at CRC treatment, showed that in the dNTPase-depleted cell line, cell proliferation was enhanced and the expression levels of the apoptosis-related proteins were decreased, presenting the functional role of SAMHD1 in treatment sensitization for 5-FU[200].

2.15.4 The golden example of cytarabine

As mentioned earlier, SAMHD1 has the potential of hydrolysing the active metabolites of NAs, thus preventing them from perturbing nucleic acid metabolism and allowing uncontrollable cell proliferation to endure in cancer cells[223]. Cytarabine, a nucleoside analogue of cytidine, has been used for leukaemia treatment since the 1960´s and to date it is characterized as part of the gold standard in AML treatment[224]. SAMHD1 recognizes the active form of cytarabine, ara-CTP, as a substrate but not as an activator and hydrolyses it, thus rendering it unable to be incorporated into the genomic DNA[80,217,225]. One of the first studies to elaborate the impact of SAMHD1 in antimetabolite treatment in

cancer therapy was from Herold et al. In a panel of 138 hematopoietic and lymphoid tissue derived cell lines, they showed that there was a direct correlation between low *SAMHD1* levels and better response to ara-C[80]. By depleting SAMHD1 expression with two mechanistically different approaches (Vpx treatment and *SAMHD1* KO) in a monocytic cell line (THP-1), the half-maximal effective concentration (EC_{50}) value for ara-C decreased by 130-fold[80]. With a similar approach, in a panel of 13 different AML cell lines, with varying range of SAMHD1 protein expression levels, using three different functional experimental procedures, a significant correlation between treatment toxicity of ara-C and SAMHD1 expression was shown. Higher protein levels led to treatment resistance and accordingly expansion of intracellular ara-CTP pools is found in SAMHD1 KO cell lines. Cells cultured continuously in low doses of ara-C can acquire resistance to treatment, but ara-C resistant cell lines treated with Vpx, were resensitized to treatment, signifying the role of SAMHD1-based resistance[217]. Treating primary adult and paediatric AML blasts with Vpx before ara-C increased treatment sensitivity[80]. It should be stressed that the example of ara-C and the success in AML treatment based on the presence or absence of SAMHD1 is cell type specific and cannot be universally applied, as in ALL cell lines, no difference in response to cytarabine treatment could be monitored[222].

In both orthotopic and heterotopic mouse models, mice that were initially injected with *SAMHD1* KO cells followed by ara-C treatment presented prolonged survival in comparison to the mice that were injected with the *SAMHD1* expressing counterparts[80,216,226]. Furthermore, in a cohort of 150 adult AML patients, SAMHD1 levels were inversely correlated to CR, where patients with high SAMHD1 levels did not reach CR. Levels of SAMHD1 can be highly predictive for EFS and relapse free survival (RFS) as these rates were lower in patients with higher SAMHD1 levels[217]. Similar to these results, in two cohorts, one including adults with *de novo* AML and one paediatric AML, there was a direct correlation between ara-C treatment response and low *SAMHD1* levels, which was translated into better OS and EFS. It is of great interest that SAMHD1 levels were irrelevant to the outcome of AML induction therapy but on the other hand patients treated with HDAC in consolidation therapy might benefit more[80]. This could be probably reasoned by the fact that during induction therapy, anthracyclines are used in combination with ara-C, and maybe they affect the outcome of patients with low SAMHD1 levels. This leads to the assumption that SAMHD1 could be used as a biomarker for treatment outcome in post-remission therapy[183].

It should be of note, that even though low SAMHD1 levels correlate with better clinical outcome in AML in ara-C treatment, this is not universal for all cancer types. SAMHD1 expression did not shape the outcome of OS or PFS in MCL patients that were included in two different protocol treatments that cytarabine was part of it[195]. Regardless of the treatment protocol, lower expression of SAMHD1 did relate to better OS[192].

2.15.5 Repurposing drugs

Understanding SAMHD1 mechanism in depleting dNTPs and NAs, opened a new window of screening candidate molecules and already used drugs from different pathological conditions, into using them against other diseases. One example of this was cancer drug clofarabine, which in phorbol-12 myristate-13 acetate (PMA) - differentiated monocytes presented a 22-fold enrichment against HIV-1 infection, when SAMHD1 was expressed[214]. In MDM cells, a variety of NAs and anti-folate drugs that are currently used for cancer treatment were tested for determining their antiviral potential. Vpx-mediated SAMHD1 KO cells improved HIV-1 inhibition for some drugs (cladribine, clofarabine, nelarabine) and impaired it for some others (capecitabine, floxuridine, fluorouracil), showing that presence of SAMHD1 in antiviral treatment could have either a beneficial or a detrimental outcome. Addition of CDK4/6 inhibitors, such as Palbociclib, enhanced the phenotype even more, however this effect could be under the influence of other cell cycle dependent proteins, and SAMHD1 should be phosphorylated in advance[227].

2.16 Identifying SAMHD1 inhibitors

From the first discoveries where SAMHD1 had the capacity to decrease dNTP levels and affect the replication of viral genetic material, the question arose whether SAMHD1 could be targeted with drugs[228]. So, SAMHD1 inhibition could have both a scientific, as well as a clinical potential. Furthermore, the many alternative possibilities of inhibition due to the large number of dNTP binding pockets made it a preferable target. One such molecule to be synthesized was 5´-methylene dUTP, which could bind to AS2 and inhibit tetramerization, thus making the enzyme inactive. However, its efficacy was restricted in *in vitro* applications due to poor cell permeability[229]. A study by Hollenbaugh et al, using computational modelling, proposed different clinically relevant compounds that could be utilized by SAMHD1 either as substrates or inhibitors based on the different modifications at the 2´-position of the sugar moiety of the respective NAs and the position of the individual residues that are located in close proximity to the catalytic pockets of the protein[225]. The significance of this study could be translated into whether approved drugs for different pathological conditions can be used as activators or substrates by SAMHD1 and affect treatment outcome directly, under the assumption however, that these findings could be verified *in vivo*[225]. According to this study, it was later speculated that CNDAC could perform as a SAMHD1 inhibitor. On the contrary, SAMHD1 expression presented intrinsic resistance and used this cytosine analogue as a substrate instead of being an inhibitor. To support that, crystal structures showed that CNDAC could not bind to AS1 or AS2 but only at the CS, hence presenting no inhibitory properties[230].

A high-throughput assay not depending on coupled-enzyme properties identified 8 small molecules as potential inhibitors of SAMHD1. This assay was based on the hydrolytic activity of SAMHD1; however, it was not established if the molecules could act as

substrates or inhibitors for SAMHD1 by binding to the alternative sites. What is of great importance though and these results should be interpreted carefully, is that the inhibitory potential was evident when dGTP was used both as an activator and a substrate, and not dCTP, which could be explained that the inhibitory effect could only be present upon structural conformations that might appear upon dGTP binding. So, these small molecules might not bind at the AS or the CS but at a different protein site.[231]. Another high throughput assay that screened 69000 small molecules, was not able to detect any of them that could act as a potential SAMHD1 inhibitor as none of them presented strong inhibitory results and could be used at a biological setting[232].

2.17 The "double-edged" sword

One of the hallmarks of cancer is the ability to evade proliferation control[233]. For cancer cells to replicate, they need an accessible intracellular dNTP pool, so the role of SAMHD1 in depleting expanded pools and hindering cancer progression is of great importance. In the absence of SAMHD1, the homeostatic mechanism of the cell can be deregulated, and this can lead to a series of problematic events. Increased dNTP pools and imbalanced dNTP ratios will be the first to be observed. This could be followed by decrease in the efficacy of DNA repair, probably increase in the number of somatic mutations and eventually all these events will lead to tumorigenesis. Hence, SAMHD1 plays a very important role as a tumour suppressor. Furthermore, regarding use of chemotherapy, low or no SAMHD1 expression in proliferating cells could potentially increase the competition between NA-TPs and canonical dNTPs for incorporation in the newly synthesized DNA molecule. This can lead to the need of increased doses of chemotherapy in order to overcome this SAMHD1-mediated resistance barrier which could potentially increase toxicity, causing this compound not to be very effective and thus suitable for treatment from a clinical perspective.

On the other hand, SAMHD1 expression can be detrimental in the efficacy of a specific group of NAs that are use at chemotherapy. If that NA is a SAMHD1 substrate, its treatment potential will decrease as SAMHD1 will present a resistant mechanism. The only way to overcome this obstacle, could be with the use of a SAMHD1 inhibitor as a combination treatment that could sensitise cells to chemotherapy. Finally, in many pathological conditions, SAMHD1 mutations have been reported. This is an additional factor to be acknowledged when applying chemotherapy as mutated residues in the binding pockets of SAMHD1 or at adjacent areas could affect drug efficacy.

All in all, even though SAMHD1 inhibition could be beneficial for chemotherapy treatment, advantages and disadvantages should be considered, for the best possible outcome, when chemotherapy treatment is to be assessed[226].

3 Research aims

The overall aim of this thesis was based on the insight that even though many antineoplastic cytotoxic therapies have led to an improved survival and more favourable prognosis for many different types of cancer, there is still the need for improving targeted therapies for many patients. We believe that better understanding of cancer drug metabolism and resistance mechanisms could lead to:

- Development of prognostic and diagnostic biomarkers
- Stratification of patients that could have a better or worse response to a specific therapeutic regimen, with the opportunity of modifying ongoing treatments
- Readjustment of treatment doses upon evaluation of initial response and the sequencing profile of expressed proteins in each patient
- Sensitization to treatment by targeting and inhibiting proteins that contribute to nucleotide metabolism and alter the efficacy of antimetabolites that follow the same activation pathway

The above aims have been evaluated with SAMHD1 as the focus, as it has potential to act as a biomarker for specific cancers where it could also be used as a target to improve therapy effect.

4 Ethical considerations

My PhD projects focus on translational research and our main goal was to evaluate ongoing therapies and discover new, more efficacious ones, that could be brought from the lab bench to the clinic. Following the preliminary results of each study acquired by *in vitro* experiments on immortalized cancer cell lines, we would eventually proceed to evaluate them initially *ex vivo*, in samples collected from patients suffering from the respective malignancy or *in vivo*, by using an animal model trying to mimic the circumstances around our hypothesis.

Use of animal models is thoroughly planned with focus not only on research aims but also on animal well-being. An ethical application is mandatory to describe the reasoning for this experiment and what is the benefit from it. All procedures followed are according to the rules and guidelines at the animal facilities and everything was stated in detail and approved beforehand in the ethical application. Animal experiments are performed only because at present no other alternative is available to recapitulate the complex pharmacokinetic properties of a drug used in an organism as well as the interplay of cancer cells with their host. The welfare of all animals was always the highest priority and to ensure optimal well-being, animals were housed in a controlled setting with environmental conditions being constantly observed, food and water being always supplied, and a variety of different additional equipment was always included for the mental stimulation of the animals. All animals were regularly monitored, and all procedures including health checks were recorded comprehensively. Everybody working with animals has been trained by qualified personnel and acquired a certificate before coming into contact with the animals. For procedures we were not very familiar with, as well as for any questions that may have risen during the process, assistance and advice from qualified personnel was always asked, to minimize any signs of pain and discomfort and in general any unpleasant conditions. We effectively applied the 3R principles (refinement, reduction, and replacement), as our experiments were always preceded by small pilot experiments for establishing the most efficient experimental conditions while enrolling the least animals possible. Since we studied haematological malignancies, which are systemic diseases, we relied on tail vein injections, for cells to circulate in the blood stream and with that in mind future applications for replacing animals with *in vitro* practices, such as 3D organ printing may not be very efficient for this line of research.

In order to be able to work with patient samples, an ethical application needs to be submitted. It needs to be reasoned why we want to perform experiments on these samples, what will be the nature of these experiments and what is the benefit that rises from our hypothesis. All patient samples used for our *ex vivo* experiments, were collected from biobanks where the patients had already given their consent for using this material in future experimental research. Furthermore, cells from healthy donors are also used as a control, so also these donating individuals should be similarly aware about the research

and the importance of written consent. Use of patient samples could be as close to testing your new treatment compounds hypothesis as possible. However, it should always be considered that it is only the pathological cells that are being examined and any probable interactions with other cell types inside the body are excluded from the research. Furthermore, trying to establish a relation between protein expression and treatment response is of great importance, so inclusion of patient samples upon information about expression of different proteins and previous or ongoing treatment is noteworthy for the research outcome.

Finally, as our ultimate goal in the present thesis was to verify whether our results can be verified and applied in clinical practice, the role of the patients enrolled in a phase I clinical trial is vital for the future of this research. They need to be thoroughly informed about the new treatment regimen and the risk of any potential side effects. It needs to be explained to them based on what criteria they have been chosen to participate and what could be the benefit for them to be enrolled in this trial and not follow other existing treatments protocols. Moreover, it needs to be clear that this is the first step of a clinical trial and there is a risk of unpredicted risk toxicity. From my perspective, this is the most integral part when doing translational research and admission of patients should be very well designed and very thoroughly explained. Obtaining consent from patients should only occur after every aspect of the study has been explained and every risen question has been clearly answered. Finally, it should be clearly stated that at any given point, the patient has the possibility to withdraw from the ongoing study without any repercussions.

5 Materials and methods

5.1 Phase 1 clinical study design

The aim of this study was to evaluate whether HU could be added in an ongoing standard treatment protocol and if there was any beneficial outcome towards its efficacy and feasibility. Patients enrolled in this trial should have been older than 18 years of age, fit for intensive chemotherapeutic treatment and newly diagnosed with non-promyelocytic AML. The study was overviewed by the centre for clinical cancer studies at Karolinska University Hospital and the treatment protocol was reviewed by both institutional and national ethics boards (Dnr 5.1-2019-4650). The treatment protocol was established as follows: ara-C was administered twice per day, on days 1-5 for 2 to 4 cycles, depending on the patient. The anthracycline daunorubicin was administered only on cycles 1 and 2, once per day during days 1-3. HU was always administered 1 hour prior to ara-C and the dose was intensified in a 3+3 design. Inclusion of additional compounds occurs only in the event of diagnosed mutations, so patients with *FLT3*-mutated AML also received midostaurin twice per day, on days 8-21 of each cycle. All patients provided a written informed consent in advance before treatment initiation and the study was conducted according to the Declaration of Helsinki. Primary endpoints of the study were safety and permissiveness after each treatment cycle. Secondary endpoints were based on response to treatment based on European LeukaemiaNet (ELN) criteria and guidelines, accumulation of ara-CTP after the first treatment cycle and MRD levels.

i. MRD measurement

MRD levels were measured either with flow cytometry following the second treatment cycle or with real-time quantitative polymerase chain reaction (RT-qPCR). Sensitive deep sequencing after cycle 2 was also used to confirm MRD levels in patients that did not have a standard validated genetic marker.

ii. Pharmacokinetic study

During the first day of the first treatment cycle, HU was administered to patients 1 hour before cytarabine infusion. Patients were divided into three groups, and they were administered either with different doses of HU or they were administered at a different infusion. Administration in one of the two infusions occurred only to exclude a possible accumulation of ara-CTP which could lead to a bias towards ara-CTP levels in the analysis and could be interpreted as an overestimation of HU efficacy. Upon treatment, peripheral blood was extracted from the patients, mononuclear cells (MNCs) were isolated and ara-CTP levels were measured as described at the respective section.

iii. *Ex vivo* drug sensitivity and ara-CTP measurements

AML MNCs were also collected and cultured *ex vivo* and were further tested for cell viability rates in combination treatments with ara-C and HU or dF-dC, and ara-CTP measurement after treatment. Both technique protocols were as mentioned in their respectively described sections. Culturing conditions differed depending on the assay. For the ara-CTP measurements cells were cultured in IMDM medium supplemented with 10% heat-inactivated foetal bovine serum (FBS) and 1% penicillin – streptomycin (PenStrep) and a combination of 4 different cytokines, GM-CSF, IL-3, IL-7 and thrombopoietin (TPO) (all were at a concentration of 20ng/ml apart from TPO that was 100ug/ml). Regarding the drug sensitivity assays, cells were cultured in RPMI-1640 medium, supplemented with 10% heat inactivated FBS and 1% PenStrep and 12.5% of conditioned cell culture supernatant from confluent HS-5 cell line.

5.2 Primary blasts culture

Experiments with primary Paediatric and adult AML blasts, as well as blasts derived from T-ALL patients were approved by the regional ethical review board in Stockholm (no.03-810, no.02-445 and no.2013/1248-31/4). Informed consent was acquired for all samples.

i. AML-derived cells

For **paper I**, cells were thawed and cultured in a dilution of 1 million cells per ml, in StemPro-34 medium supplemented with StemPro nutrient supplement, 10% heat-inactivated FBS, 1% PenStrep and a combination of 4 different cytokines: GM-CSF, IL-3, IL-7 and TPO. All cytokines were resuspended in the medium in a 20ng/ml concentration. At a 24h time-point, $10x10^6$ cells were collected and resuspended in 2ml of the supplemented medium. In a 24-well plate they were equally distributed and treated either with 50ul of empty virus-like particles (VLPs) (control) or 50ul of Vpx-VLPs, to knock down SAMHD1 expression. After 3 hours of incubation at the same conditions as described at the human cell culture section, all cells treated with the same type of VLPs were pooled together and resuspended in a $0.5x10^6$ concentration and incubated overnight. The following day, 15000 cells were plated in each well, on a white 384-well plate.

ii. T-ALL cells

In paper II, cells were thawed and cultured in IMDM medium supplemented with 10% heat-inactivated FBS, 1% PenStrep and 25ng/ml recombinant human IL-7. Cells were incubated for 4 hours in a $2x10^6$ cells per ml concentration in the supplemented medium and then diluted in $1x10^6$ cells per ml concentration and incubated overnight. The following day 12500 cells were dispensed in each well on a white 384-well plate.

5.3 Patient sample collection and therapeutic analysis

A group of 222 *de novo* AML patients that had tissue samples collected and were available for immunohistochemical evaluation of SAMHD1 protein expression were enlisted in the **paper IV** study. All samples had been collected by two different institutions: the University of Texas MD Anderson Cancer Center (MDACC) and the National University Hospital of Singapore (NUH). From all abovementioned patients BM specimens were collected and out of those, 189 had validated immunohistochemical results. The research use of all samples was according to the Declaration of Helsinki, and it had been approved by the Review Boards of the respective institutions. Both groups presented a heterogeneous image in regard to SAMHD1 protein expression in AML blasts, treatment protocols and cytogenetic risk groups. This study was purposely designed this way for a better understanding and validation of the importance of SAMHD1 expression in a clinical setting.

i. Tissue microarray construction and immunohistochemistry

For the generation of the TMAs **in paper IV**, tissue was obtained in duplicates from tumour areas that were rich in AML blasts and all samples were collected preceding induction therapy of the patients. Two different types of controls were used for this method, paraffin embedded tissue blocks of cell lines and tissue sections from reactive BM specimens. All analyses were performed at the same laboratory following the same staining protocols for consistency, and stainings were performed using an automated detection system. To verify SAMHD1 expression in normal hematopoietic cells, double stainings of SAMHD1 and specific markers expressed in those cells were applied. In summary immunohistochemistry technique was performed through the following steps. First, the slides containing the samples were deparaffinized and rehydrated. Then samples were treated with antigen epitope retrieval to allow the antibody to detect the protein of interest. Samples were then incubated with blocking buffer to minimize unspecific binding, followed by overnight incubation with the primary antibody. After washing to remove the excess antibody, detection of the primary antibody occurred by an HRP-bound secondary antibody which was finally detected by DAB (3,3´-diaminobenzidine) chromogen. Samples were then counterstained with haematoxylin and eosin, dehydrated with increasing concentrations of ethanol solution, and they were mounted before assessment under the microscope. In **paper III** different double stainings were performed at diagnosis and at remission. In both papers the evaluation of the results was blind and a total of at least 500 blasts were included per sample by two haematopathologists independently. The final value of protein expression was determined as a percentage of SAMHD1 positively stained blasts in relation to the total number of blasts.

5.4 Orthotopic mouse models

Two mouse models were used in **paper I.** All animal experiments and procedures were carried out according to the rules and guidelines formed by the Swedish Board of Agriculture. All practices were stated at the ethical applications #N89/14 and 5718-2019 and were approved by the regional ethical committee. Mice were housed in a continuously monitored controlled environment, with food and water always at their disposal. Experimental planning and execution always followed the "3R" principles and there were always pilot experiments performed towards refinement, with the welfare of the animals being always at the frontline of our considerations. Female NOD/SCID (non-obese diabetic / severe combined immuno-deficiency) mice were injected with human cells expressing SAMHD1 or their SAMHD1-deficient generated clones diluted in PBS, intravenously at the tail. Mice were randomly assigned to treatment groups and 6 days post cell injection, drug treatment was initiated with the assigned compounds being administered intraperitoneal, according to the protocol referring to their respective group. Disease progression was monitored via a bioluminescence imaging system by injecting intraperitoneally D luciferin. All experimental and humane endpoints were registered at the ethical applications and were also governed by established regulations of the facility. One major consideration regarding these experiments is treatment toxicity. Even though based on the preliminary data from the pilot experiment that should be prevented, if any animal died within a timeframe of two weeks from treatment starting point, it was considered due to treatment toxicity since onset of leukemic symptoms had not yet appear, so the animals were censored. During autopsy all abnormalities were recorded, and samples were collected for further examination.

Another mouse model used for the study at **paper I** was CD45.2 C57BL/6J. All procedures were approved by the local ethical committee (ethical application #1869). *MLL-AF9* murine cells were injected in the tail vain of the animals and 20 days after cell injection, mice were injected intraperitoneally with the compounds that were assigned in their respective groups, with doses and time of injection, stated at the protocols. Read-out of leukemic burden and disease progression was monitored by flow cytometry and a haematology analyser. Experimental and humane endpoints of the experiments were appointed as mentioned above in the orthotopic mouse model injected with human cells.

5.5 Human cell line culture

A variety of different human cell lines have been used for these studies. Among these, for the AML studies our main model was a childhood AML cell line called THP-1. This cell line expressed high levels of SAMHD1, and this was our first line of conclusions after testing new compounds or performing functional experiments. Regarding the T-ALL project, two cell lines were used; MOLT-16 and Sup-T11, a childhood and an adult T-cell acute lymphoblastic leukaemia cell line respectively. All cell lines were cultured in IMDM medium,

supplemented with 10% heat inactivated FBS and 1% PenStrep and they were incubated in a humidified incubator at 37°C with 5% CO_2 concentration. All cell lines are suspension cells, so upon splitting, cell concentration was between 0.3 and 0.5 million cells per ml. Cells were regularly tested for mycoplasma contamination by commercially available kits. SAMHD1 deficient cell lines were generated with the use of two different protocols. Regarding THP-1 and MOLT-16 cell lines, exon 1 was targeted with gRNAs and for Sup-T11 exon 2 was targeted.

i. VLP production

For **papers I and II**, Virus like particles (VLPs) containing Vpx were used *in vitro* to deplete SAMHD1 expression and evaluate the difference of NA treatment in the presence or absence of SAMHD1. VLPs were produced by transfecting HEK293T cells either with plasmids encoding VLPs containing Vpx, or with plasmids encoding VLPs absent of Vpx as a control. After 24 hours, cell culture medium was changed with fresh, and 48 hours post change, the supernatant was collected and filtered. The collected supernatant was afterward purified through sucrose and the product was then resuspended in 300μl of RPMI-1640 medium, supplemented with PenStrep. All VLPs were quantified and normalized based on a modified qPCR assay[80].

ii. Compound preparations

All compounds used at **papers I and II** were directly diluted in dimethyl sulfoxide (DMSO) in stock solutions with higher concentrations than the ones used for the experiments and stored at -20°C. Regarding all working solutions, compounds were diluted into lower concentrations before plating, avoiding this way repeating freezing – thawing cycles of the higher concentration stock vials that could lead to diminished efficacy of the drugs.

iii. Phenotypic screening

For **paper I**, in order to verify potential SAMHD1 inhibitors, a phenotypic screening was performed using 4 different compound libraries. Compounds were placed in white 384-well plates in 10mM concentrations diluted in DMSO. Cytarabine was used as a positive control and DMSO was used as a negative control. Plates were heat-sealed and stored at -20°C until further use. Before adding THP-1 SAMHD1 proficient cells, plates were equilibrated at room temperature for 30 minutes and mildly centrifuged before removing the seal. With the use of a multidrop device, 1000 cells were added in each well, and all plates were incubated in a plastic chamber for 72 hours, with wet paper placed at its inner side walls to prevent evaporation of the cell samples. Plates were removed from the incubator and equilibrated at room temperature (RT) for 30 minutes before adding the luminescent cell viability assay and then cell viability was measured with a plate reader.

iv. Proliferation inhibition assays and drug synergy analysis

For **papers I and II,** with the use of a digital dispenser, compounds were distributed in serial dilutions in white 384-well plates, with the range of dilutions fluctuating depending on compound and cell line. Furthermore, by using the synergy wizard software of this machine, matrices of combinations between two different compounds could be designed for analysing potential synergistic or antagonistic interactions among them. The values from the wells with cultured cells treated with high concentrations of the compounds tested were used as a control for 0% cell viability. On the other hand, the values from the wells where cells were treated only with 1% DMSO were used as a 100% cell viability control. All the other results were normalized as a percentage of cell viability based on the range created from these two values. DMSO was used as a control since all drugs were diluted in DMSO for being compatible to use at the digital dispenser, and all wells were normalized to 1% DMSO concentration. Depending on the cell line and their respective doubling time, different cell numbers were plated, and the endpoint of the experiment could also differ. All plates were incubated in a humidity chamber in the humidified incubator to minimise evaporation. Plates were removed from the incubator and equilibrated at room temperature for 30 minutes before adding the luminescence cell viability assay and measuring signal intensity via a plate reader. All measurements were analysed using a four-parameter logistic nonlinear regression model in Prism GraphPad software.

Combination treatment results obtained from the luminescence assay were analysed with the online Synergy Finder application. By using a zero-interaction potency (ZIP) model, an average synergy score was calculated to determine synergistic (delta score > 0), antagonistic (delta score < 0) or zero interaction (delta score =0) effects among the compounds. Delta scores of > 5 denote strong synergy[234].

5.6 *In silico* analysis of RNA correlation with drug sensitivity

For **paper II**, we investigated the correlation between *SAMHD1* and *ADA* RNA expression in T-ALL cell lines against sensitivity to different compounds including nelarabine. Two different datasets were downloaded from the DepMap portal. The first dataset was the RNA expression data for protein coding genes from the Cancer Cell Line Encyclopaedia (CCLE)[235]. It was released through the fourth quarter of 2022 (22Q4) and contained 1408 cell lines and 19193 genes. The second dataset comprised of drug response data including the area-under-concentration (AUC) response curve sensitivity scores from the Cancer Therapeutics Response Portal (CTRP)[236,237] for 1107 cell lines and 545 chemical compounds and was released in 2015. Expression data for *SAMHD1* and *ADA* were plotted against the AUC data of nelarabine using R statistical software (v 2022.12.0+353)[238]. Plots were generated using the ggplot2 package[239], with Pearson's correlation and the P values calculated using the stat_cor() command.

5.7 Laboratory molecular biology techniques

i. Enzyme-coupled SAMHD1 activity assay

With the use of an enzyme-coupled malachite green biochemical assay, the enzymatic activity of SAMHD1 against different NAs and the canonical dNTPs, as well as the likelihood that some NAs can act as allosteric activators was validated (**papers I and II**). SAMHD1-mediated dNTP hydrolysis produces dNs and inorganic triphosphates. These triphosphates can be used as a substrate by PPase and produce inorganic phosphate, which in turn is measured by an absorbance assay performed by a plate reader[211,212]. A standard curve of inorganic phosphate was used to measure the inorganic phosphate produced by the PPase. Background signal was initially subtracted, and phosphate released from the SAMHD1 reaction was calculated using the equation created from the phosphate standard curve. Substrate-velocity curves were fitted using the Michaelis-Menten model and GraphPad software was used for results visualization. Recombinant SAMHD1 protein and *E.coli* PPase was produced by Protein Science Facility at Karolinska Institutet.

ii. HPLC-MS/MS assay for intracellular dNTP and NA-TP measurements

The intracellular levels of the active metabolites of the NAs as well as the levels of the individual dNTPs were measured in **papers I, II and III.** Cells were treated with the respective compounds, in sub-lethal doses and they were incubated for the indicated time. After incubation, $2x10^6$ cells were collected and after being washed with DPBS, they were immediately resuspended in 65% ice cold methanol and placed on ice. Samples were vigorously vortexed, followed by heating at 95°C. After centrifugation the supernatant was collected and was vacuumed dried. The amounts of intracellular dNTPs and NA-TPs were measured using ion pair chromatography tandem mass spectrometry. Results were verified by using student unpaired t-test with Prism GraphPad software.

iii. Western blot analysis

Western blot is one of the most trivial techniques regarding protein levels visualisation. Many different protocols and alterations have been developed; however, the sequence of the different steps remain standard. First, the protein cell lysate is separated with gel electrophoresis and then the proteins are transferred from the gel to a membrane. With the use of a primary antibody against the protein of interest and then with a secondary antibody against the species the primary was raised, the presence or absence of a protein can be established, as well as its relative expression levels. Expression levels can be visualized either by a direct fluorophore at the secondary antibody or via enhanced chemiluminescence (ECL) via detecting the conjugated horseradish peroxidase (HRP) enzyme at the secondary antibody. In **papers I and II**, through western blotting, protein

expression levels of SAMHD1 in the parental cell lines or after different treatment protocols were established, as well as SAMHD1 depletion of the knockout clones. Furthermore, expression of apoptotic markers upon treatment was also validated with this technique. Finally, estimation of SAMHD1 levels in primary cells was quantified including the change in levels followed by Vpx treatment.

iv. Cellular thermal shift assay

In **paper I**, we were interested to determine whether the potential SAMHD1 inhibitors bind directly to the protein and affect its activity either by destabilizing the enzyme or by occupying the catalytic site, thus preventing dNTP or NA-TP binding. This binding can be easily viewed by CETSA through a shift in the melting curves of a protein, since direct binding, increases that melting temperature[240]. Following compound treatment, cell pellets were collected, resuspended in a standardized cell number to lysis buffer ratio and placed in PCR tubes. After heating each tube in a different temperature, samples were equilibrated at room temperature, followed by three freeze-thaw cycles and vigorous vortexing at the end of each cycle. Cell lysates were collected after centrifugation and the melting temperature of the samples was validated by western blot.

v. *In situ* chemical cross-linking

The dNTPase activity of SAMHD1 is directly correlated with its ability to form very stable homotetramers. So inevitably **in paper I**, incubating SAMHD1 with numerous different compounds for inhibiting its phosphatase ability, raised the question of whether these compounds act through dissociation of the homotetramer. After treating the cells with individual compounds, they were washed, aliquoted and each aliquot was resuspended in a solution of decreasing concentrations of a cross linker. The cross linker was prepared in anhydrite DMSO and diluted in PBS to reach the decided concentration. After 30 minutes of incubation, the reaction was quenched with Tris-HCl, and samples were centrifuged and processed for protein visualization with western blot. Cross-linking techniques allow us to distinguish complex formations from sole proteins according to molecular weight separation and based on the molecular weight of the protein of interest, it can be seen whether it is present as a monomer or if it is bound to other monomers or other proteins.

6 Results and discussion

Paper I: Identifying RNR inhibitors as SAMHD1 inhibitors and improving treatment outcome in AML through combination treatment with cytarabine.

As SAMHD1 expression can be used as a predictive biomarker for cytarabine efficacy in AML, enhancing its pharmacological potential could be favourable for patients. Several studies have tried to discover a compound that could inhibit SAMHD1 activity, but even though preliminary results *in vitro* showed promising inhibitory potential against recombinant SAMHD1, they were not effective in cells. Towards this direction, we applied a phenotypic screen of more than 33000 different small molecules in order to discover potential SAMHD1 inhibitors.

Our strategy was based on two central pillars. Firstly, cell proliferation inhibition by these molecules should be SAMHD1-dependent. So, all molecules were tested combined with a non-toxic ara-C concentration in both parental THP-1 cells and their SAMHD1-deficient clones, and the ones that had an effect independently of SAMHD1 presence were excluded. Secondly, the compounds should illustrate this inhibitory effect only when combined with ara-C. So, they were tested both in presence and absence of ara-C, and the ones that had a toxic effect independent of ara-C presence were also excluded. As a final step, we performed experiments with serial dilutions in both SAMHD1 proficient and deficient cell lines, both with and without ara-C (**figure 5**). Out of all the positive hits, we focused on gemcitabine (dF-dC), a clinically approved deoxycytidine analogue. Gemcitabine had been previously discovered to initiate two different regulatory roles regarding nucleotide metabolism. When diphosphorylated (dF-dCDP), it acts as a non-allosteric RNR inhibitor[241,242], and when triphosphorylated (dF-dCTP) it stalls DNA replication[243]. Furthermore, dF-dC, even though it is a NA, it is not a SAMHD1 substrate, so it's activity could not be compromised by SAMHD1[80].

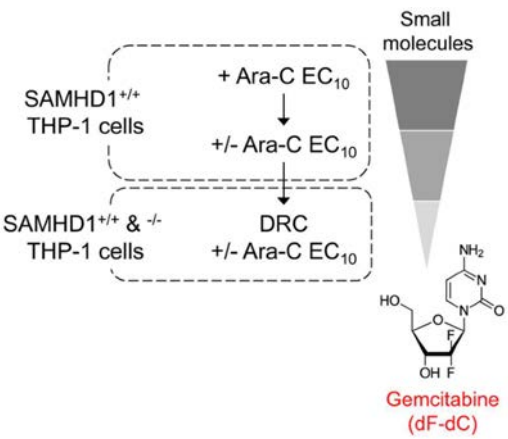

Figure 5. SAMHD1-inhibitor screen: Schematic representation of the methodology that was followed for the high-throughput phenotypic screen of more than 33000 small molecules that led to the identification of gemcitabine as a SAMHD1 inhibitor.

(Source: Rudd SG, Tsesmetzis N, Sanjiv K et al, **Ribonucleotide reductase inhibitors suppress SAMHD1 ara-CTPase activity enhancing cytarabine efficacy,** *EMBO Mol Med. 2020 Mar 6;12(3):e1049. doi: 10.15252/emmm.201910419. Epub 2020 Jan 17.)*

Based on our initial experiments that included cellular thermal shift assay (CETSA) and *in situ* chemical cross linking we extrapolated that this inhibition was not due to direct binding of the compound to the enzyme. If dF-dC directly bound on the SAMHD1, this interaction might change the thermostability of the protein and would change the aggregation temperature (T_{agg})[244], however in this case, T_{agg} temperatures were similar, arguing against a direct binding between the drug and the protein. Furthermore, with the *in situ* cross linking assay, we were able to determine that the SAMHD1 tetramer structure levels remained unaltered upon treatment.

It was previously described that the diphosphorylated dF-dC is an RNR inhibitor (RNRi)[241]. RNR is an allosterically regulated enzyme, and it is responsible for catalysing the conversion of NDPs to dNDPs and is a rate limiting enzyme for the *de novo* dNTP synthesis[126]. Since dF-dC did not directly bind to SAMHD1, we hypothesized that more RNR inhibitors could affect SAMHD1 activity and two more allosteric inhibitors of RNR, hydroxyurea (HU) and triapine (3-AP), were confirmed to present the same results[245]. as addition of either of these two compounds, sensitized cells to ara-C treatment. For all 3 compounds, their ability to sensitise to ara-C treatment was directly correlated to SAMHD1 protein levels. Cell lines that expressed higher SAMHD1 levels showed a bigger shift towards ara-C treatment sensitization. Moreover, they all presented synergistic results in SAMHD1-proficient cells treated with combination treatments of ara-C and RNRi based on ZIP-scores, a feature that was not seen in SAMHD1-deficient cells. ZIP-score is a developed method that compares the dose response curves that are generated from single drug treatments with the combination treatment and can validate the synergistic or antagonistic potency of the drug combination. This is based on the assumption that if both compounds do not interact with each other, then there would be small changes in the respective dose response curves. Then, based on the difference form this zero interaction, a delta score is calculated to measure the combination treatment potency. Furthermore, in a panel of 9 haematological cell lines, a direct significant correlation between treatment synergy and SAMHD1 protein levels was evident ($p=0.0045$ for HU and $p=0.0311$ for dF-dC). Also, by using the same CETSA and *in situ* cross linking techniques, we verified that HU was also not directly bound to SAMHD1.

The synergy between RNRi and ara-C was also confirmed with experiments monitoring DNA damage and apoptotic markers. Single drug treatment of sublethal doses of ara-C had no effect on them, but combination treatments with RNRi induced DNA damage and cell death. This was evaluated through Western blotting, and it was indicated by an observed increase in expression levels of apoptotic marker PARP and DNA damage markers pChk1, pChk2 and γH2Ax when cells were treated with a combination treatment, but there was no change in levels when cells were treated with either of the two compounds in single treatment. Furthermore, addition of RNRi did not affect anthracycline

efficacy, which was of great importance since ara-C is used in combination with anthracyclines in induction therapy.

With this data, our group was the first one to show SAMHD1 inhibition at living cells. Following these results, we wanted to verify if this SAMHD1-dependent barrier could also be relieved *in vivo*. We performed our *in vivo* experiments by testing two of the discovered SAMHD1 inhibitors. Due to low cost, as well as the long precedence in AML treatment, HU was chosen as one of the RNRi to be used for this study. In two orthotopic mouse models where two different cell line models were used, combining ara-C with HU, significantly prolonged the survival of the mice in a SAMHD1-dependent fashion. In the first mouse mode, NOD/SCID mice were subjected to tail injections with THP-1 or HL60 wild type cells expressing SAMHD1 or their SAMHD1 depleted counterparts, and 6 days post cell injection they were split in four different treatment groups where they were administered with PBS, ara-C alone, HU alone or a combination of ara-C and HU for five consecutive days. All cells were expressing firefly luciferase so the leukemic growth could be monitored through an imaging system. No differences were observed in the control groups treated with PBS with respect to survival. Interestingly, in mice injected with SAMHD1 proficient THP-1 cells, treatment with ara-C showed no different effect compared to the PBS-treated control group, but in SAMHD1 deficient mice, survival was prolonged significantly (from a median survival of 50 days at the control mice to 68 days in this group, p=0.0018). Furthermore, in mice injected with wt THP-1 cells, combination treatment increased median survival at 64 days (p=0.0141, compared to the ara-C single drug treatment). These results were confirmed when testing the efficacy of the treatment on HL-60 injected cells. Furthermore, these results were recapitulated with the use of the other RNR inhibitor, dF-dC. The experimental protocol was similar as before, but due to toxicity issues, dF-dC was administered only two times, once on day 1 and once on day 3 while ara-C and PBS was administered for 5 days. Animals treated with PBS, ara-C alone and dF-dC single drug treatment displayed similar median survival between 44 and 49.5 days. However, combination treatment prolonged median survival to 65 days (p=0.0014 and p=0.0097 compared to ara-C and dF-dC single drug treatments respectively). It should be noted that in both models, transient loss of weight was shown.

Because in xenograft models, mice need to be immunocompromised to accept human cells, we used another additional model where we injected *MLL-AF9* murine cells in CD45.2 C57BL/6J mice, to also monitor the development of any BM toxicity. These cells express SAMHD1 and were intravenously injected at the mice. Twenty days after injection mice were divided in 4 groups and were treated with normal saline, ara-C, HU or a combination treatment. This is a very aggressive AML model and median survival for each of the groups treated with normal saline, ara-C, HU or a combination treatment was 6, 12, 8 and 14 days respectively. Significance in survival was reached when comparing the combination treatment with the control group (p=0.0026) but it was not detected when

comparing the ara-C or the HU treated groups with the control group. Once more, combination treatment prolonged survival in a SAMHD1-based manner and furthermore, myelotoxicity was not increased compared to the single drug treatment. The latter was of great importance since both HU and ara-C create myelotoxicity, and increased toxicity could render this combination treatment highly toxic. Unchanged myelotoxicity levels were detected by comparing blood markers via flow cytometry one day after the conclusion of chemotherapy or by assessing spleen size and BM cellularity at sacrifice.

Combination treatment efficacy was also shown *ex vivo* in both paediatric and adult AML samples with varying expression of SAMHD1 protein levels. A correlation was established between SAMHD1 protein expression levels and response to combination treatment base on the ZIP-synergy scores (p=0.0466), as increasing treatment concentrations of the RNR inhibitors, sensitized cells more to ara-C treatment. Furthermore, in most of the samples where SAMHD1 was depleted with Vpx treatment followed by either single drug or combination treatment, response to ara-C remained similar, showing that in the absence of SAMHD1, no sensitization to ara-C treatment was observed when adding HU. On the contrary, upon addition of RNR inhibitors, sensitization to ara-C was observed in cells expressing SAMHD1 incubated with control VLPs, with EC_{50} values, decreasing up to a magnitude of two. Finally, comparison of the ZIP-synergy scores between the Vpx-VLP treated and the control treated groups, illustrated a significant decrease in synergy of the two compounds in the absence of SAMHD1 (p=0.0046) suggesting that this drug combination is effective and acts synergistically in a SAMHD1-dependent effect manner

All results pointed out that SAMHD1 activity is affected by RNR inhibitors. However, the actual mode of action was not clear. Many studies have shown that post translational modifications occur in SAMHD1 and influence its catalytic capability. However, functional experiments excluded SAMHD1 phosphorylation or tetramerization inhibition as the reason behind the enzyme's activity depletion.

When inhibiting RNR, intracellular dNTP levels decrease, and the cell cycle can be affected. As mentioned before, SAMHD1 has been discovered to be regulated and phosphorylated by CDKs in a cell cycle dependent manner. Also, phosphorylated SAMHD1 has been shown to have a lower efficacy when the intracellular dNTP pools are low. To that end, when expressing either phosphomimetic T592E SAMHD1 mutant or a mutant that cannot be phosphorylated (T592A SAMHD1) in SAMHD1-depleted THP-1 cells, we did not detect any difference in the synergistic potential of the RNRi with ara-C, indicating that it is not phosphorylation that affects SAMHD1 ability.

Instead, the cause of SAMHD1 deregulation was linked to intracellular dNTP pool imbalance. Treatment with dF-dC or HU did not affect either the homotetramer formation as shown by CETSA experiments or its proportions as was depicted the *in situ* cross linking experiments. Treating cells with low concentrations of RNR inhibitors resulted in an

alteration of the dNTP ratios, where dCTP levels increased after treatment, thus altering the dCTP to dATP ratios by 3- to 6-fold, a finding that was not observed when treating the cells with ara-C single drug treatment. This increase in levels, led dCTP into binding at a higher rate to AS2. This change in ratios led to a reduced ara-CTPase capacity of the homotetramer and subsequent increase of ara-CTP levels by 4-fold. These levels were equally relevant to SAMHD1 negative THP-1 cells.

Finally, we wanted to detect how this elevated concentration leads to inhibition of ara-CTP hydrolysis. To that end, recombinant SAMHD1 was incubated with GTP that can only bind at AS1, ara-CTP that can bind at the CS of SAMHD1 and increasing concentrations of non-hydrolysable dNTPαS that can bind at AS2. It should be noted that dNTPαS can also bind at SAMHD1 CS, but they cannot be hydrolysed. Even though 3 out of these 4 dNTP analogues (dTTPαS, dGTPαS and dATPαS) did not affect SAMHD1 capacity to hydrolyse ara-CTP, when dCTPαS was used ara-CTP hydrolysis could not be initiated, even in concentrations 20- to 100-fold higher compared to those needed by the other 3 analogues to initiate ara-CTP hydrolysis. However, the homotetramer could still be assembled since incubation of SAMHD1 with dCTP could activate its dCTPase ability.

Finally, since RNR inhibition affects the *de novo* synthesis pathway of dNTPs, we tested whether it is the salvage pathway that could be involved in this occurrence of dNTP ratio imbalance. Supporting this hypothesis, quantification of phosphorylated dCK in serine 74 residue, which marks the activated form of the enzyme showed increased levels of phosphorylated dCK between 8- and 20-fold depending on the RNRi treatment.

To summarize, with this study we were able to establish an indirect SAMHD1 inhibition at a cellular level and we were able to do so with the use of RNR inhibitors including dF-dC and HU. More importantly, HU is already used in the clinic for AML patients with known toxicity effects and its cost is not a limiting factor for introducing it in treatment regimens. Our preclinical findings presented strong results regarding the efficacy of combining HU with ara-C and becomes a candidate treatment for being able to be tested at a clinical setting in a small cohort. Furthermore, SAMHD1 could be introduced as a predictive biomarker for personalized medicine in AML patients.

Paper II: SAMHD1 inhibition sensitizes T-lymphoblastic acute leukaemia to nelarabine.

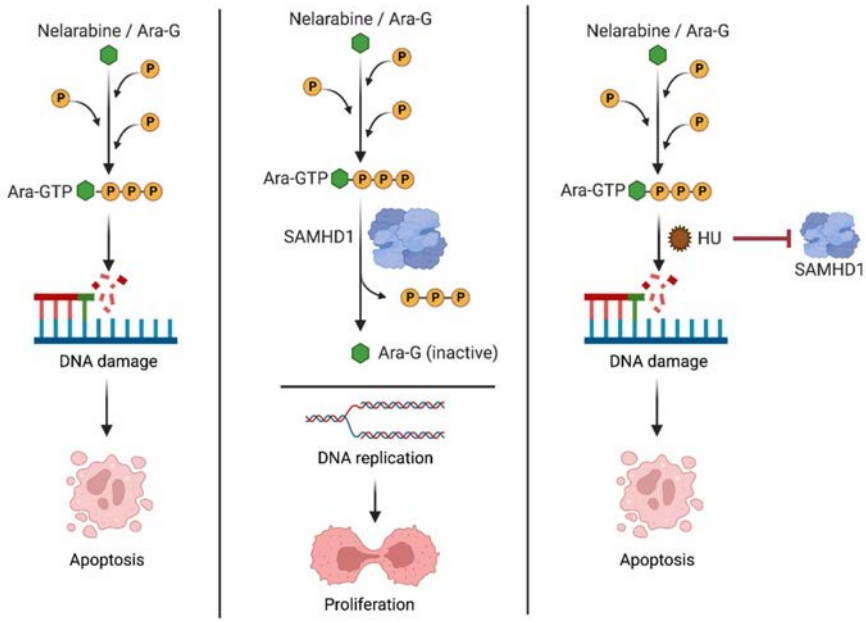

Figure 6. Graphical representation of SAMHD1's negative effect in nelarabine/ara-G treatment. After ara-G is phosphorylated into the active metabolite of ara-GTP, it can incorporate into the elongated DNA strand, stop the DNA replication process and subsequently lead the cell to apoptosis. SAMHD1 expression can recognise ara-GTP as a substrate at the CS and cleave ara-GTP into ara-G and PPPi, thus promoting resistance to treatment. Addition of HU as a combination treatment, inhibits SAMHD1, thus sensitising cells to nelarabine/ara-G treatment, which will lead the cell to apoptosis. (*Image created with Biorender)*

Following our findings in AML, we hypothesized that SAMHD1 expression could have an unfavourable impact in another haematological malignancy, T-ALL. An earlier study showed in a panel of various T-ALL cell lines, that upon nelarabine treatment, two distinct groups could be identified. One that was resistant and one that was sensitive to treatment[246]. Our initial hypothesis was that this divergence could be possibly related to SAMHD1 expression, and it was verified by testing the levels of two cell lines that belonged in each respective group. Later, another study verified that T-ALL cells respond better to nelarabine treatment compared to B-ALL, which was also explained by the fact that T-ALL cell lines largely express less SAMHD1[222].

Through this study, via an enzyme-coupled assay, we were able to identify that ara-GTP belongs to the category of NAs that can be both an allosteric regulator and a substrate for SAMHD1. Inorganic triphosphate was detected when SAMHD1 was incubated with ara-GTP and GTP. However, since GTP can only bind at AS1 but not AS2, this could only lead to the assumption that ara-GTP can also occupy the space in the AS2 pocket and initiate

the formation of the active homotetramer and thus its own mediated hydrolysis. Also, with ara-GTP being a guanosine analogue, it was not very surprising that it had the capacity to bind at AS1 as well. This introduces an additional problem about nelarabine treatment in T-ALL. Since ara-GTP can bind at AS2 it would compete with physiological dNTPs for that position thus needing higher concentrations to be applied for treatment. Also, binding at AS2 activates its own hydrolysis, which means that compared to drugs that can only bind at CS, relatively higher concentrations of the drug should be administered for the cells to be sensitized to treatment. So, that increase in concentration could render the drug unavailable for treatment due to possible toxicities. In simple terms, due to inducing its own hydrolysis, ara-GTP might be even more affected by SAMHD1 resistance as compared to ara-CTP.

As mentioned earlier, the active metabolite of nelarabine is ara-GTP, however ara-G is not water soluble, which renders its application more complicated. Drugs used for patient treatment are diluted either on saline or water, but in the case of ara-G this was not feasible, so without a solution to this problem, use of ara-G would not become possible for patient treatment. For this reason, the pro-drug nelarabine was developed, which is water soluble and through the enzymatic activity of adenosine deaminase (ADA), it is demethoxylated into ara-G and subsequently phosphorylated via sequential steps to ara-GTP. In standard cell culture protocols, the use of heat-inactivated serum reduces ADA activity to a great extent. So, if the cell line used for *in vitro* experiment testing does not express ADA, a lack of conversion to ara-G will reduce its efficacy. Experimentally, this can be circumvented by using ara-G instead of nelarabine, as cells will be probably more sensitized to treatment[247]. Most probably this observation should not have relevance while performing *in vivo* experiments as most animals should express ADA. However, it should point out the limitations and differences that can occur when one performs experiments in such a controlled environment as testing a cell line *in vitro*, and extrapolates results based on them for following steps. In one of the cell lines used for this study, ADA was not expressed and there was a difference in response to nelarabine compared to ara-G. More specifically in ADA-deficient cells, even in high concentrations of nelarabine the cell line remained resistant to treatment, while treatment with ara-G at the same concentration range, showed response. In comparison, an ADA expressing cell line presented similar IC_{50} values between nelarabine and ara-G treatment. Both compounds for these *in vitro* studies were diluted in DMSO, which is a limitation of this *in vitro* technique and cannot be applied for future *in vivo* procedures. We broadened this search into more cell lines, and in a group 10 T-ALL cell lines that were analysed *in silico*, there was a significant negative correlation between *ADA* mRNA expression and sensitivity to nelarabine treatment based on the area-under-the-curve (AUC) values ($p=0.0072$). Furthermore, there was also a correlation between resistance to nelarabine and *SAMHD1* mRNA expression in T-ALL cell lines ($p=0.02$), but this correlation could not be reproduced in AML cell lines ($p=0.28$).

Among the few cell lines that express SAMHD1, two of them, one of paediatric and one of adult origin, were chosen for further functional analyses. Depletion of SAMHD1 in both cell lines with two different approaches (VLP-Vpx treatment or CRISPR/Cas9) resulted in sensitization to treatment, thus rendering SAMHD1 as a resistance factor towards nelarabine treatment in T-ALL. Treatment with Vpx sensitized MOLT-16 and Sup-T11 cell lines by decreasing the IC_{50} values by 2-fold and 40-fold respectively. Similarly, SAMHD1 KO via CRISPR/Cas9 editing sensitized cells to treatment by 10-fold and 100-fold respectively based on the IC_{50} values. Of note, Vpx treatment only incompletely decreased SAMHD1 expression, however it was sufficient to increase treatment sensitivity.

Based on our previous studies in AML, we were able to show that addition of HU can indirectly affect SAMHD1 enzymatic activity and sensitize cells to ara-C treatment. SAMHD1 expressing cells were slightly more sensitive to HU treatment. When combined with nelarabine or ara-G, it led to a HU dose-dependent sensitization to treatment compared to single drug treatment in both T-ALL parental cell lines that expressed SAMHD1 by up to 10-fold for MOLT-16 and 5-fold for Sup-T11. This was not observed in the SAMHD1-deficient cell lines. Furthermore, to support our findings, we measured ara-GTP levels in all cell lines, and we were able to identify vastly larger levels of ara-GTP in the non-expressing SAMHD1 cell lines compared to their wild type counterparts. On top of that, addition of HU in the SAMHD1 proficient MOLT-16 and Sup-T11 cell lines, significantly increased ara-GTP levels by 3-fold ($p=0.0402$) and 2-fold ($p=0.0007$) respectively, showing that HU-mediated inhibition of SAMHD1 sensitizes cells to treatment. On the contrary, this increase in ara-GTP levels was not observed in SAMHD1-depleted cells ($p=0.9461$ and $p=0.5321$ for MOLT-16 and Sup-T11 respectively).

Adding HU to a combination treatment with nelarabine, also showed a sensitization to nelarabine treatment in *ex vivo* T-ALL patient samples. All samples were chosen based on *SAMHD1* mRNA expression. However, because previous studies had shown that SAMHD1 promoter can be methylated and decrease partially or completely protein expression[222], in parallel with the cell proliferation assays, cells were collected and SAMHD1 protein expression was also validated. The IC_{50} values of nelarabine in these five samples ranged from 0.1μM to 29μM, however similar to our results from the AML study, the protein levels of SAMHD1 and IC_{50} values acquired from nelarabine treatment were not correlated[80]. This could be explained probably by the small number of samples used for this experiment, but most likely indicates that comparing IC_{50} values across primary cells is difficult due to interpatient variability. Finally, addition of HU sensitised cells to nelarabine and reduced the IC_{50} values by a factor of 1.2 to 2.2, suggesting that even at a moderate amount, inhibition of SAMHD1 still occurs in primary T-ALL cells thus rendering nelarabine treatment more efficacious.

Taking everything into account, it appears that SAMHD1 can be used as a predictive biomarker for nelarabine treatment in T-ALL, as higher protein expression levels increase resistance to treatment. This is of outmost importance since nelarabine is an important drug for treatment in patients with R/R T-ALL[248]. Addition of RNR inhibitors such as HU, appear to remove this SAMHD1-based barrier and sensitise cells. Combination treatments of nelarabine and HU can be of great importance, since lower levels of nelarabine would be able to have the desired outcome without the risk of treatment related side effects such as neurotoxicity that have been reported in some cases[178,249].

Paper III: Newly diagnosed AML patients enrolled in a phase 1 clinical trial combining hydroxyurea with cytarabine achieved complete remission.

As mentioned earlier, OS for AML patients is below 30% but this number is highly dependent on the patient's age, as older people have a dramatically worse prognosis due to intolerability of intensive chemotherapeutic regimens[161]. The backbone of AML treatment consists of cytarabine and anthracyclines. and although various studies have included additional compounds to improve treatment efficacy, most of them had negative results, with the only exceptions being FLT3 inhibitors[250]. We wanted to validate whether addition of HU to standard AML treatment is beneficial and safe. Eligibility criteria for inclusion in this study were that all patients should be newly diagnosed with non-promyelocytic AML, all patients should be over 18 years of age, and all patients should be competent to tolerate intensive chemotherapy. During this trial, all patients received at least two full cycles of chemotherapy, with the only exception being one patient that due to age, the second cycle was 80% of the initial first cycle dosage.

Addition of HU resulted in no additional severe toxic side effects in any of the patients. Regarding observed toxicities due to treatment, it is notable to mention that only haematological grade 3/4 toxicities were reported. Furthermore, haematological recovery was not delayed by HU-induced SAMHD1 inhibition as shown by the recovery of neutrophil values to pre-treatment values within a median time of 19 days (range 16-23) after the first cycle of chemotherapy and the recovery of platelet values after within a median time of 22 days, which shows that addition of HU does not significantly increase haematological toxicity. No non-haematological grade 3/4 toxicities were observed.

Based on the treatment protocol, HU was given to patients in increasing doses in a controlled manner comprised of three steps, with the first one being at 500mg twice per day, the second one being one dose of 1000mg and one of 500mg and the last step being at 1000mg twice per day. According to the results that no toxicities related to increased HU dosages were observed, the highest dose at step 3 was established as the recommended dose for the upcoming phase 2 trial that will register more patients. It

should be noted however, that dose titration to dose-limiting toxicities was not a goal for this phase 1 trial.

All monitored patients in this phase 1 trial achieved CR. Eight of them achieved it already after the first cycle and the ninth patient following the second cycle of chemotherapy. Regardless of the readout technique used to assess MRD, eight evaluable patients, were all negative for MRD after the second treatment cycle. No measurements were obtained for the remaining patient because they did not have the specific leukaemia associated immunophenotype (LAIP) nor a marker suitable for PCR-based MRD assessment. Based on the already published data retrieved from the Swedish AML registry, patients that received the same treatment regimen as with this study but without the inclusion of HU had rates of CR and MRD negativity at 92%/80% for the favourable risk group, 84%/64% for the intermediate risk group and 71%/60% for the adverse risk group of patients. On the contrary in this study all patients in this phase 1 trial achieved CR and presented MRD negativity. So, compared to this data, although this phase 1 study, is small in number, it showed promising results and enhanced effectiveness on both CR and MRD negativity. With the follow up phase 2 study, these promising results might become more solid[251].

In BM biopsies that were collected from the patients at the time of diagnosis, SAMHD1 protein levels were established with IHC. Patients expressed a wide range of protein levels, and based on this expression they were clustered in three groups, therefore categorized as low, intermediate, and high. In the absence of HU, SAMHD1 expression and ara-CTP levels were not correlated *in vivo* (n=9, p=0.83). Similarly, SAMHD1 expression and ara-C IC_{50} values were not associated *ex vivo* (n=7, p=0.13). As we would expect a correlation between SAMHD1 and ara-C, in both of these experimental procedures, lack of correlation could be explained by the small size of samples that has been used.

Finally, Inclusion of 500mg HU in the treatment did not alter ara-CTP levels in PBMCs (n=3, p=0.45), however, doubling the dose to 1000mg significantly raised levels to a median of 150% (n=6, p=0.04). Besides, increase in ara-CTP measurements was also detected in *ex vivo* BM-derived blasts after HU treatment compared to the ara-C single treatment (n=8, p=0.02). This was also illustrated in BM-derived mononuclear cells where IC_{50} values of ara-C decreased upon adding HU for combination treatment by a median value of 2.1 (p=0.0047). These results were also supported by combination treatment with dF-dC, another RNR inhibitor, where ara-C IC_{50} values decreased by a median factor of 1.6 (p=0.01). Verification of these results also occurred with the calculation of ZIP scores that define whether a combination treatment is synergistic or not, and in this case, they were similar with the THP-1 control measurements.

All in all, based on this small phase 1 trial it appears that addition of HU could increase the efficacy of cytarabine when administered as a combination treatment. This could be helpful in paediatric cases of AML and in adult AML where the patients are fit for intense

chemotherapy. Treatment tolerance was one of the criteria for enrolment in this trial. Older patients however, over the age of 70, where OS rates are the worst usually are unfit for treatment and cannot tolerate intensive chemotherapy. So, with adding HU to the current treatment protocol, even though our results presented no additional toxicity effects, it could still be detrimental for the health of these frail patients. Many factors can be taken into account to find a solution towards a better outcome for these patents. Initially, SAMHD1 could be used as a biomarker, and based on our data, cells expressing higher levels of SAMHD1 react better to HU inclusion. So, there might be a potential in adjusting the dose of ara-C by decreasing it, which could partially alleviate the intensity of the current treatment. This could also be validated at future studies, by monitoring the half-life of ara-CTP, as sequential measurements of ara-CTP could allow dose adjusting in patients and it will also allow to establish how long patients will be exposed to ara-CTP.

Paper IV: Establishing a correlation between low SAMHD1 expression and cytarabine treatment in AML patients.

Even though there has been a great improvement in AML treatment, prognosis remains unsatisfactory as there are still many patients that will succumb to their disease. This bad prognosis is age related, as the OS rates in paediatric AML is greater than 70%, while in adults it is around 25% only, thus making the mortality rate of AML patients one of the worst compared to other cancer types[252]. New treatment protocols in induction therapy for paediatric AML have shown CR rates over 90%, but still 1 out of 3 children might still relapse with the OS rates then decreasing to approximately 30%[253,254]. Besides, our group and others had already shown that in AML, SAMHD1 could potentially act as a biomarker, since there was an inverse correlation between its expression levels and cytarabine efficacy in a variety of cell lines and SAMHD1-depletion could reverse the effect of ara-C treatment resistance and sensitize cells *in vitro*, *in vivo* and *ex vivo*[80,217].

In two *de novo* AML cohorts that were collected from two different institutions, the University of Texas MD Anderson Cancer Center (USA) and the National University Hospital of Singapore, we evaluated SAMHD1 expression and whether there is a correlation between expression and cytarabine therapy efficacy, both following induction therapy and consolidation therapy. With immunohistochemistry (IHC), we were able to identify that SAMHD1 stainings were negative in the normal hematopoietic stem cells and progenitors, but on the contrary, all AML blasts were positive for SAMHD1 expression, with a median and a mean value being 30% and 42% respectively and with a range of 1% to 100% for SAMHD1-positive blasts per sample. So, presence of SAMHD1 in myeloblasts could be directly considered as a pathological finding.

As expected, levels of SAMHD1 were not correlated to the administered ara-C dose during induction therapy. Based on the frequency distribution, a cut-off was introduced to

distinguish three different groups depending on *SAMHD1* expression. Based on visual examination of the SAMHD1 expression, low *SAMHD1* was established at a 25% cut-off whereas high *SAMHD1* was determined by a 75% cut-off. The remaining blasts were considered as intermediate.

After induction treatment, no association could be established between *SAMHD1* expression and CR achievement, either when all patients were analysed as a complete group (p=0.76) or when analysing the subgroup of patients that received HDAC at consolidation therapy (p=0.32). Furthermore, there were no differences detected in CR in patients that received ara-C based treatment as induction therapy according to *SAMHD1* expression in either of the two cohorts from both institutions (p=0.77 for MDACC and p=0.12 for NUH respectively). Notably, there was a discrepancy among the two cohorts in the percentage of patients that accomplished CR (85% in MDACC vs 21% in NUH), which might be explained by recruitment bias and more intensive induction therapy at MDACC.

While no differences with respect to SAMHD1 expression were evident during induction treatment, 5-year EFS in patients with low *SAMHD1* was higher with 47% compared to the 20% for the group of patients expressing high *SAMHD1* (p=0.0086). Similarly, 5-year OS was 44% for patients with low *SAMHD1* compared to 34% for patients expressing high levels of *SAMHD1* (p=0.0114).

The lack of effect during induction treatment probably stems from the potent contribution of anthracyclines to therapeutic outcome. As SAMHD1 does not confer resistance to anthracyclines, SAMHD1's role for overall outcome of induction therapy is limited. However, during consolidation therapy without anthracyclines, SAMHD1 appears to impact outcomes of ara-C. So, since anthracyclines are only part of induction and not consolidation treatment, the effect of SAMHD1 on a treatment that only contains NAs, such as HDAC, is expected to be greater as SAMHD1 does not affect outcome of anthracyclines.

7 Conclusions and future perspectives

Since the discovery of SAMHD1, numerous studies have been conducted regarding its regulation, known functions, and possible therapeutic applications. SAMHD1 has been shown to, among other functions, adjust intracellular dNTP pools, participate in cell cycle progression, restrict viral infections, and assist in DNA repair. It has also become apparent that SAMHD1 is an important player in cancer treatment as in many cancer types it is found to be mutated, deregulated, or post-translationally modified. This survival mechanism obtained by cancer cells could have been developed since expression of wild type SAMHD1 could negatively affect tumour progression. Contrary to that, SAMHD1 expression can be a regulator of cancer treatment potential, especially when NAs are included in the chemotherapeutic protocols. In this thesis, we tried to shed some light in mechanistic and functional aspects of SAMHD1, the interactions with other proteins and enzymes in the cell cycle and the nucleotide metabolism pathway. We also tried to decipher whether SAMHD1 can be assigned the role of a prognostic biomarker, initially for AML and in the future for other types of malignancies, as well as if it could be targeted for developing treatment protocols of personalised medicine.

- We were able to establish SAMHD1 as a predictive biomarker for AML progression and showed a strong correlation between SAMHD1 expression and event-free survival and overall survival rates in patients treated with high doses of cytarabine during their consolidation therapy.
- We were the first to discover small molecule inhibitors that could be applied at a cellular level and inhibit SAMHD1 activity via an indirect but effective manner. Also, we demonstrated the SAMHD1-mediated resistance barrier to cytarabine can be overcome by inhibiting SAMHD1 in AML.
- Survival was prolonged *in vivo* in a SAMHD1-related way in animal models treated with combination treatment of RNR inhibitors and cytarabine.
- Imbalances of the dNTP pool play an important role towards function and efficacy of SAMHD1. Depleting SAMHD1 expression in AML and T-ALL cells can increase intracellular concentrations of the active metabolites of cytarabine and nelarabine respectively.
- Implementing hydroxyurea in the treatment protocol of newly diagnosed AML patients did not exhibit any additional side effects. All enrolled patients achieved complete remission and MRD was below detection levels. *Ex vivo* analysis from patient derived samples developed an increase of ara-CTP levels both in PBMCs as well as in BM derived cells.

Following the initial results obtained from the phase 1 clinical trial, it became apparent that the highest applied dose of HU did not show any additional or unexpected side toxicity effects while it showed highest increase in the ara-CTP concentration levels. These

results, however, should be validated in a phase 2 study, where a larger number of patients will be enrolled, and this high HU dose will be the established dose. Furthermore, for a more precise evaluation of this synergistic effect and for the development of more targeted therapies, it should be beneficial to have more than one ara-CTP measurement. This could allow to determine the half-life of the circulating drug, adjust, and refine the optimal dose as well as have a better assessment towards clinical responses.

During the phenotypic screening, more compounds were positive hits as potential SAMHD1 inhibitors. These hits should be followed up with mechanistic studies, to verify whether they have an inhibitory effect towards SAMHD1. If this is confirmed, then is should be examined if they can directly bind to it, which could be more efficacious since they could either prevent enzyme tetramerization or substrate binding at the catalytic site. Furthermore, as in the case of RNR inhibitors it would be of great importance to determine any other relations among the enzymes involved at nucleotide metabolism.

SAMHD1 appears to have a similar role in developing treatment resistance in T-ALL and RNR inhibitors seem to have a similar effect in SAMHD1 enzymatic activity depletion. This study should be followed up, if possible, with *in vivo* studies to verify whether SAMHD1 expression affects nelarabine efficacy and based on that to validate if RNR inhibitors can prolong survival in animals in a SAMHD1-dependent manner.

Finally, regarding AML, cytarabine appears to be the chemotherapeutic drug that in combination with hydroxyurea or gemcitabine can drastically improve prognosis and survival. In T-ALL though, as nelarabine is the only compound that has been approved for R/R patients, more studies should be conducted with potential candidate drugs against T-ALL that could have a better outcome in the absence of SAMHD1.

8 Acknowledgements

First, I would like to express my gratitude to Karolinska Institutet, for providing me with a high-class research environment, in which I had the opportunity and honour to interact and collaborate with talented researchers through my doctoral studies.

There are not enough words to express my gratitude to my principal supervisor, **Nikolas Herold**. First of all, you believed in me and gave me the opportunity to work with you in the field of cancer research that I wanted so much. Not only are you a brilliant medical doctor and researcher, but you are also the best supervisor a student could have. You have always been there to guide me but at the same time you allowed me to be independent and formulate my own ideas and hypotheses. You have been an excellent role model, with the ability to positively motivate everyone around you and I hope I fulfilled your expectations. But you have also been there as a friend. In our meetings, except for work, you would always care to ask how I have been doing. I will always have the best memories of all the times we spent together at work, during conferences but also outside of work. Finally, I know that I am our first PhD student, but I am sure that there will be many, many, many more in the future.

Jan-Inge Henter, my co-supervisor, I would like to thank you for all your guidance and support throughout my studies. You have always been there to assist me, and you would always try to make some time for me. Being invited at your "frihetsfest" was a great honour and just being at the same room with you is motivating to continue doing research. You always treat everybody as an equal and you would always listen to and take into consideration everybody's opinion during our group meetings. You have been a real group leader and if I ever come to a similar position as you, you will always serve as the best example.

My co-supervisor, **Georgios Rassidakis**, you have been guiding me from my first steps as a researcher. You were always guiding me and helping me to develop new ideas through our meetings and our scientific discussions we have had through these years. You would always come with new hypotheses, and you always inspire me.

Sean Rudd, my co-supervisor, during my initial days as a doctoral student we would spend a lot of time working together for our projects. I have gained a lot of knowledge from you during those days. You were patient to teach me many different techniques, but you also taught me the importance of carefully planning and optimizing my working days at the lab. Every day you would find time to discuss with me my progress and my results and this was very valuable for a doctoral student who was taking his initial steps. You were always there to support me, and you always reminded me not to hesitate to contact you if I had any questions.

My co-supervisor **Magda Lourda**, I would like to thank you for all the guidance and advice you have been giving during my studies. You always assisted me with your expertise in immunology and many related techniques and during our meetings you always tried to answer all the questions I might have.

Ingrid Lilienthal, I don't even know how to start thanking you for just being you. You are the unicorn of the lab. For quite some time it was just the two of us in the lab and it felt as if we were a dream team! Whenever I had a question, you were the first person I would go to. Regardless of how tired you were or how much work you had; you would always find some time to listen to what I have to say. Always with a smile and always with patience. You have taught me so many things in the lab that I now know that made me a better researcher and a better person. Thank you!

Frida Holm, I would like to express my gratitude for all the help you have provided through my studies. You would always give me positive and constructive feedback after my presentations and during our meetings, you would always come up with ideas to drive our research forward.

Anette Langebäck, your organisation skills are undisputable. You constantly contribute to creating a better work environment for everyone. In all our meetings, you would always keep notes about all our projects and then through discussions, with your experience you would always help us move the projects forward.

Hala Habash, the little one! You have been helpful from day one. Whenever you had a few minutes of free time, you would always come and ask if we need anything. You are a never-ending source of good energy and joy, always smiling and it is always great to be around you! With our office desks being next to each other, you always created a very friendly work environment. I will always remember all the scientific discussions we had as we were discussing our projects, over a jar constantly full of candy. You are at the beginning of your scientific journey, but you have already achieved so much. I am sure that you will have a bright future!

Agnes Sorteberg, the calm force! Somehow, you always manage to be around when someone might need assistance with their experiments. You are one of the kindest people (and probably one of the clumsiest) I have ever met. I really enjoyed the meetings we had all the times we had to troubleshoot and optimise different technique protocols. I always knew that if I ever needed any assistance at the lab, without hesitation you would help me. Your growing experience and your assistance during our *in vivo* studies have been invaluable.

Silvia Angori, it feels that I have known you since we were children. There was no adjustment period for us to establish a good working relation. You radiate positive energy from the first day we met! You always see the glass half-full; you are friendly and with a

great sense of humour, but at the same time hard working. I will always have this image of you moving everywhere carrying your protocols and for every question one might have, there is always a piece of paper with the answer we are looking for.

Rakan Naboulsi, thank you for your help and guidance in the field of bioinformatics. Your experience in many techniques has always been helpful and you would always assist me with scientific input in my studies.

Magda Barbachowska, thank you for your assistance in our projects. I enjoyed every minute of our discussions about troubleshooting and optimising technique protocols.

Eva and **Borja**, you showed from the first day the potential you have for a bright future in the field of research. You are very organised when planning and when presenting during our meetings it seemed as if you had years of expertise. I am happy to have worked with you and I hope during our time together, I was able to teach you some of the things I know.

Sabina Enlund, I really enjoyed all the scientific conversations we had in our small, clustered area with the office desks. Trying to optimise technique protocols and figure out how to optimise the schedule with the bookings at the hood at the cell lab was a never-ending conversation. But apart from science, we also had some very funny conversations, some of which I will always remember with a smile on my face.

Lilly Velentza, my toastmaster. You are a breeze of fresh air. With our office desks being adjacent we had such a great time with so many good laughs. Apart from being a great friend, you are also a great researcher and you always tried to assist whenever you had the opportunity.

Cecilia Dominguez, you make our working environment a better place! You constantly try to assist everyone, and you always help us with lab related tasks. But apart from work, you have also been a great friend through my studies. Always there to listen to what I had to say, and you would always try to assist if possible. **Jennifer Frithiof**, you have the ability to make everyone around you laugh. You create a very good atmosphere and a very friendly work environment. I would never miss out the opportunity to have a lunch break with you.

I would also like to express my gratitude to **Anna Nilsson**, **Kees-Jan Pronk** and **John-Inge Johnsen**, members of the committee at my half-time seminar, for their constructive feedback and advice they provided, and helped me for the remaining of my studies. **Caroline Rådestad**, thank you for all your administrative help through my studies. **Désirée Gavhed**, thank you for all your help and for always caring to learn about my progress.

Femke Harteveld, thank you for all the nice moments and the encouragement. You are a very good friend, and you would always try to assist in every possible way. **Abishek Arora**,

when you are around it is certain that we will have a great time. Thank you for sharing your enthusiasm, happiness, and joy with the rest of us.

Jan-Bernd Stukenborg, you would always show interest in my research, and you would always try to help me progress in my studies with your advice and your expertise. I would also like to thank you for all the joyful moments we have spent at the lab because of your great sense of humour.

Phil Newton, **Kristiina Tammimies**, **Klas Blomgren** and **Adamantia Fragkopoulou**, I would like to express my gratitude for all the advice and feedback you have given me through meetings or when we just met and at the office space and we had scientific discussions. You always try to make our working environment better for everyone working at the floor.

Giannis Zerdes, a true friend and a brilliant researcher. Your field of knowledge is so broad that I am always looking forward to having scientific discussions with you, but also discussions about football. It was a great honour to name me as your toastmaster at your dissertation. You and Lilly were the first people to invite when I moved to a new apartment, and I will always remember that you had to build your own chair to sit.

Francesca Mastropasqua, you are the person I have known the longest within my work environment. You are always patient to answer to all my questions. I am always looking forward to your dinner invitations and an extra slice of focaccia. Hopefully, you will get better at board games and be able to beat me at some point.

Jonathan Sohl, my mentor, thank you for all the conversations we have had and for all the advice you have given me throughout my studies.

Christina and **Anastasia**, you have been great friends since the first days that we arrived at Uppsala for our master programmes. We may not see each other as often as we would like to, but I am always looking forward to meeting you for a fika! **Sander** and **Alberto**, I am always looking forward to meeting with you and have some fun.

To everybody at our floor at J9:30, for creating a great work environment and for knowing that we all work as a big group, and whenever someone needs assistance, everybody will try to help. **Linnea, Jessica, Dawei, Dorina, Angela, Yifan, Yiqun, Hajar, Rageia, Yanhua, Joao, Eva, Twana, Xander, Yunhan, Therese, Tim, Zhengpei, Michelle, Marika, Jose, Mahtab, Alkis, Chris, Alejandro, Thalia, Carlos, Yana, Ahmed, Yara, Lil, Elena, Suzanne, Valentina,** thank you for all your kind support, your friendship and all the great memories we have created all these years.

I would also like to express my gratitude at **Malin**, **Conny**, **Velina** and **Jessica** for their assistance, help and support during my *in vivo* studies. You were always willing to help me and your assistance and guidance during my first days cannot be described in words in how beneficial was for me. I will always follow your advice for the rest of my career.

I would also like to thank all my friends involved at our board game groups. I was always looking forward to spending time with you in such a relaxing, yet competitive manner. **Dennis, Christoffer, Lars, Jonathan, Joel, Henrik, Daniel** and **Martin**, thank you for all the great times we had together. **Signe** and **Anna**, thank you for your friendship and for always caring to ask me how I am doing. **Sara**, you are such a kind person that always puts a smile on my face and makes me happy. **Gustav, Malin** and **Madeleine**, thank you for all the great time we had during our dinner sessions. **Tom**, thank you all your jokes, teaching me how to use my camera and for being such a great friend to Tyr and doing everything you can to spoil him.

I would like to express my gratitude to **Lejla** for always listening to what I have to say and for always giving great and very helpful advice.

I would also like to thank my friends in Greece, who I also consider them as family, and I am always looking forward to meeting them. **Roza**, you are my other half. You are the best friend anyone could ask for. **Petro M.,** becoming the best man in your wedding was a great honour. Never lose your temperament and your sense of humour. **Tasoula**, you are my oldest friend and I have only the best memories from all these years of friendship. **Fela**, you always text to see how I am doing and complain when I am not texting back. But I know you do so because you care. **Pano**, you are one of the most positive people I know, and you always try to make other people have a great time. **Nasia** and **Vaggeli,** you are two of the funniest people I know with a great sense of humour. You always know how to make us all have a good time! **Dimitra, Dimitri, Katerina, Maria K., Petro B and Maki** thank you for all your support and friendship all these years. You have always been there for me and spending time with you is a guarantee for great time. **Rouli** and **Soula,** thank you for your love and your support all these years. I don't think I will ever be able to express my gratitude for everything you have done for me! **Aki, Tzina, Maria V**. my friends from my bachelor studies. You are like a family to me and whenever we have the chance, we always try to meet up. Thank you for your support and your friendship all these years and thank you for all the great memories I have.

To my parents **Kyriako** and **Evaggelia**, my sister **Georgia**, my nephew **Kyriako**, my niece **Sarra** and my brother-in-law **Paraskeva**; words cannot express how lucky I have been to have such a caring family, for letting me follow my dreams and always support me through my journey.

Tyr, my little puppy (I am aware that you are a dog, and you cannot read this, but you need to be acknowledged). Thank you for giving me unconditional love! No matter how tired I might have been, coming home and watching you wagging your tail full of joy and jumping on me for hugs, is the solution to everything!

Robert, thank you for always being there for me. You have been unconditionally supporting me all this time. Thank you for all your patience through these years. I hope you know how much it means to me.

9 References

1. de Rooij JD, Zwaan CM, van den Heuvel-Eibrink M. Pediatric AML: From Biology to Clinical Management. *Journal of clinical medicine*. 2015;4(1):127-149.
2. Patrick K, Vora A. Update on biology and treatment of T-cell acute lymphoblastic leukaemia. *Current opinion in pediatrics*. 2015;27(1):44-49.
3. Kohnken R, Kodigepalli KM, Wu L. Regulation of deoxynucleotide metabolism in cancer: novel mechanisms and therapeutic implications. *Molecular cancer*. 2015;14:176.
4. Pai CC, Kearsey SE. A Critical Balance: dNTPs and the Maintenance of Genome Stability. *Genes*. 2017;8(2).
5. Rudd SG, Valerie NCK, Helleday T. Pathways controlling dNTP pools to maintain genome stability. *DNA repair*. 2016;44:193-204.
6. Helleday T, Rudd SG. Targeting the DNA damage response and repair in cancer through nucleotide metabolism. *Molecular oncology*. 2022;16(21):3792-3810.
7. Mathews CK. Deoxyribonucleotide metabolism, mutagenesis and cancer. *Nature reviews Cancer*. 2015;15(9):528-539.
8. Li N, Zhang W, Cao X. Identification of human homologue of mouse IFN-gamma induced protein from human dendritic cells. *Immunology letters*. 2000;74(3):221-224.
9. Liao W, Bao Z, Cheng C, Mok YK, Wong WS. Dendritic cell-derived interferon-gamma-induced protein mediates tumor necrosis factor-alpha stimulation of human lung fibroblasts. *Proteomics*. 2008;8(13):2640-2650.
10. Schultz J, Ponting CP, Hofmann K, Bork P. SAM as a protein interaction domain involved in developmental regulation. *Protein science : a publication of the Protein Society*. 1997;6(1):249-253.
11. Aravind L, Koonin EV. The HD domain defines a new superfamily of metal-dependent phosphohydrolases. *Trends in biochemical sciences*. 1998;23(12):469-472.
12. Schmidt S, Schenkova K, Adam T, et al. SAMHD1's protein expression profile in humans. *Journal of leukocyte biology*. 2015;98(1):5-14.
13. St Gelais C, de Silva S, Amie SM, et al. SAMHD1 restricts HIV-1 infection in dendritic cells (DCs) by dNTP depletion, but its expression in DCs and primary CD4+ T-lymphocytes cannot be upregulated by interferons. *Retrovirology*. 2012;9:105.
14. Martinez-Lopez A, Martin-Fernandez M, Buta S, Kim B, Bogunovic D, Diaz-Griffero F. SAMHD1 deficient human monocytes autonomously trigger type I interferon. *Molecular immunology*. 2018;101:450-460.
15. Berger G, Turpin J, Cordeil S, et al. Functional analysis of the relationship between Vpx and the restriction factor SAMHD1. *The Journal of biological chemistry*. 2012;287(49):41210-41217.
16. Brandariz-Nuñez A, Valle-Casuso JC, White TE, et al. Role of SAMHD1 nuclear localization in restriction of HIV-1 and SIVmac. *Retrovirology*. 2012;9:49.
17. Hofmann H, Logue EC, Bloch N, et al. The Vpx lentiviral accessory protein targets SAMHD1 for degradation in the nucleus. *Journal of virology*. 2012;86(23):12552-12560.

18. Schaller T, Pollpeter D, Apolonia L, Goujon C, Malim MH. Nuclear import of SAMHD1 is mediated by a classical karyopherin α / β1 dependent pathway and confers sensitivity to VpxMAC induced ubiquitination and proteasomal degradation. *Retrovirology.* 2014;11:29.
19. Du J, Peng Y, Wang S, et al. Nucleocytoplasmic shuttling of SAMHD1 is important for LINE-1 suppression. *Biochemical and biophysical research communications.* 2019;510(4):551-557.
20. White TE, Brandariz-Nuñez A, Valle-Casuso JC, et al. Contribution of SAM and HD domains to retroviral restriction mediated by human SAMHD1. *Virology.* 2013;436(1):81-90.
21. Beloglazova N, Flick R, Tchigvintsev A, et al. Nuclease activity of the human SAMHD1 protein implicated in the Aicardi-Goutieres syndrome and HIV-1 restriction. *The Journal of biological chemistry.* 2013;288(12):8101-8110.
22. Welbourn S, Miyagi E, White TE, Diaz-Griffero F, Strebel K. Identification and characterization of naturally occurring splice variants of SAMHD1. *Retrovirology.* 2012;9:86.
23. Shi Y, Lv G, Chu Z, et al. Identification of natural splice variants of SAMHD1 in virus-infected HCC. *Oncology reports.* 2014;31(2):687-692.
24. 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.
25. Franzolin E, Pontarin G, Rampazzo C, et al. The deoxynucleotide triphosphohydrolase SAMHD1 is a major regulator of DNA precursor pools in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America.* 2013;110(35):14272-14277.
26. Bonifati S, Daly MB, St Gelais C, et al. SAMHD1 controls cell cycle status, apoptosis and HIV-1 infection in monocytic THP-1 cells. *Virology.* 2016;495:92-100.
27. Goncalves A, Karayel E, Rice GI, et al. SAMHD1 is a nucleic-acid binding protein that is mislocalized due to aicardi-goutières syndrome-associated mutations. *Human mutation.* 2012;33(7):1116-1122.
28. Rice GI, Bond J, Asipu A, et al. Mutations involved in Aicardi-Goutières syndrome implicate SAMHD1 as regulator of the innate immune response. *Nature genetics.* 2009;41(7):829-832.
29. Dale RC, Gornall H, Singh-Grewal D, Alcausin M, Rice GI, Crow YJ. Familial Aicardi-Goutières syndrome due to SAMHD1 mutations is associated with chronic arthropathy and contractures. *American journal of medical genetics Part A.* 2010;152a(4):938-942.
30. Ramesh V, Bernardi B, Stafa A, et al. Intracerebral large artery disease in Aicardi-Goutières syndrome implicates SAMHD1 in vascular homeostasis. *Developmental medicine and child neurology.* 2010;52(8):725-732.
31. Thiele H, du Moulin M, Barczyk K, et al. Cerebral arterial stenoses and stroke: novel features of Aicardi-Goutières syndrome caused by the Arg164X mutation in SAMHD1 are associated with altered cytokine expression. *Human mutation.* 2010;31(11):E1836-1850.
32. Leshinsky-Silver E, Malinger G, Ben-Sira L, et al. A large homozygous deletion in the SAMHD1 gene causes atypical Aicardi-Goutiéres syndrome associated with mtDNA deletions. *European journal of human genetics : EJHG.* 2011;19(3):287-292.
33. Xin B, Jones S, Puffenberger EG, et al. Homozygous mutation in SAMHD1 gene causes cerebral vasculopathy and early onset stroke. *Proceedings of the National Academy of Sciences of the United States of America.* 2011;108(13):5372-5377.

34. du Moulin M, Nürnberg P, Crow YJ, Rutsch F. Cerebral vasculopathy is a common feature in Aicardi-Goutieres syndrome associated with SAMHD1 mutations. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108(26):E232; author reply E233.
35. Crow YJ, Chase DS, Lowenstein Schmidt J, et al. Characterization of human disease phenotypes associated with mutations in TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR, and IFIH1. *American journal of medical genetics Part A*. 2015;167a(2):296-312.
36. Adang L, Gavazzi F, De Simone M, et al. Developmental Outcomes of Aicardi Goutières Syndrome. *Journal of child neurology*. 2020;35(1):7-16.
37. Gramberg T, Kahle T, Bloch N, et al. Restriction of diverse retroviruses by SAMHD1. *Retrovirology*. 2013;10:26.
38. Goujon C, Arfi V, Pertel T, et al. Characterization of simian immunodeficiency virus SIVSM/human immunodeficiency virus type 2 Vpx function in human myeloid cells. *Journal of virology*. 2008;82(24):12335-12345.
39. Srivastava S, Swanson SK, Manel N, Florens L, Washburn MP, Skowronski J. Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable macrophage infection. *PLoS pathogens*. 2008;4(5):e1000059.
40. Le Rouzic E, Belaïdouni N, Estrabaud E, et al. HIV1 Vpr arrests the cell cycle by recruiting DCAF1/VprBP, a receptor of the Cul4-DDB1 ubiquitin ligase. *Cell cycle (Georgetown, Tex)*. 2007;6(2):182-188.
41. Goujon C, Rivière L, Jarrosson-Wuilleme L, et al. SIVSM/HIV-2 Vpx proteins promote retroviral escape from a proteasome-dependent restriction pathway present in human dendritic cells. *Retrovirology*. 2007;4:2.
42. Wei W, Guo H, Han X, et al. A novel DCAF1-binding motif required for Vpx-mediated degradation of nuclear SAMHD1 and Vpr-induced G2 arrest. *Cellular microbiology*. 2012;14(11):1745-1756.
43. Descours B, Cribier A, Chable-Bessia C, et al. SAMHD1 restricts HIV-1 reverse transcription in quiescent CD4(+) T-cells. *Retrovirology*. 2012;9:87.
44. Lahouassa H, Daddacha W, Hofmann H, et al. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. *Nature immunology*. 2012;13(3):223-228.
45. Welbourn S, Strebel K. Low dNTP levels are necessary but may not be sufficient for lentiviral restriction by SAMHD1. *Virology*. 2016;488:271-277.
46. Ruffin N, Brezar V, Ayinde D, et al. Low SAMHD1 expression following T-cell activation and proliferation renders CD4+ T cells susceptible to HIV-1. *AIDS (London, England)*. 2015;29(5):519-530.
47. Puigdomènech I, Casartelli N, Porrot F, Schwartz O. SAMHD1 restricts HIV-1 cell-to-cell transmission and limits immune detection in monocyte-derived dendritic cells. *Journal of virology*. 2013;87(5):2846-2856.
48. 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.
49. Hrecka K, Hao C, Gierszewska M, et al. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature*. 2011;474(7353):658-661.
50. Berger A, Sommer AF, Zwarg J, et al. SAMHD1-deficient CD14+ cells from individuals with Aicardi-Goutières syndrome are highly susceptible to HIV-1 infection. *PLoS pathogens*. 2011;7(12):e1002425.

51. Kim B, Nguyen LA, Daddacha W, Hollenbaugh JA. Tight interplay among SAMHD1 protein level, cellular dNTP levels, and HIV-1 proviral DNA synthesis kinetics in human primary monocyte-derived macrophages. *The Journal of biological chemistry*. 2012;287(26):21570-21574.
52. Sze A, Belgnaoui SM, Olagnier D, Lin R, Hiscott J, van Grevenynghe J. Host restriction factor SAMHD1 limits human T cell leukemia virus type 1 infection of monocytes via STING-mediated apoptosis. *Cell host & microbe*. 2013;14(4):422-434.
53. Chen S, Bonifati S, Qin Z, et al. SAMHD1 suppresses innate immune responses to viral infections and inflammatory stimuli by inhibiting the NF-κB and interferon pathways. *Proceedings of the National Academy of Sciences of the United States of America*. 2018;115(16):E3798-e3807.
54. Qin Z, Bonifati S, St Gelais C, et al. The dNTPase activity of SAMHD1 is important for its suppression of innate immune responses in differentiated monocytic cells. *The Journal of biological chemistry*. 2020;295(6):1575-1586.
55. Ahn J, Hao C, Yan J, et al. HIV/simian immunodeficiency virus (SIV) accessory virulence factor Vpx loads the host cell restriction factor SAMHD1 onto the E3 ubiquitin ligase complex CRL4DCAF1. *The Journal of biological chemistry*. 2012;287(15):12550-12558.
56. Guo H, Wei W, Wei Z, et al. Identification of critical regions in human SAMHD1 required for nuclear localization and Vpx-mediated degradation. *PloS one*. 2013;8(7):e66201.
57. Schwefel D, Groom HC, Boucherit VC, et al. Structural basis of lentiviral subversion of a cellular protein degradation pathway. *Nature*. 2014;505(7482):234-238.
58. Cassiday PA, DePaula-Silva AB, Chumley J, Ward J, Barker E, Planelles V. Understanding the molecular manipulation of DCAF1 by the lentiviral accessory proteins Vpr and Vpx. *Virology*. 2015;476:19-25.
59. DeLucia M, Mehrens J, Wu Y, Ahn J. HIV-2 and SIVmac accessory virulence factor Vpx down-regulates SAMHD1 enzyme catalysis prior to proteasome-dependent degradation. *The Journal of biological chemistry*. 2013;288(26):19116-19126.
60. Sharifi HJ, Furuya AK, Jellinger RM, Nekorchuk MD, de Noronha CM. Cullin4A and cullin4B are interchangeable for HIV Vpr and Vpx action through the CRL4 ubiquitin ligase complex. *Journal of virology*. 2014;88(12):6944-6958.
61. Hofmann H, Norton TD, Schultz ML, Polsky SB, Sunseri N, Landau NR. Inhibition of CUL4A Neddylation causes a reversible block to SAMHD1-mediated restriction of HIV-1. *Journal of virology*. 2013;87(21):11741-11750.
62. Wei W, Guo H, Liu X, et al. A first-in-class NAE inhibitor, MLN4924, blocks lentiviral infection in myeloid cells by disrupting neddylation-dependent Vpx-mediated SAMHD1 degradation. *Journal of virology*. 2014;88(1):745-751.
63. Laguette N, Rahm N, Sobhian B, et al. Evolutionary and functional analyses of the interaction between the myeloid restriction factor SAMHD1 and the lentiviral Vpx protein. *Cell host & microbe*. 2012;11(2):205-217.
64. Kyei GB, Cheng X, Ramani R, Ratner L. Cyclin L2 is a critical HIV dependency factor in macrophages that controls SAMHD1 abundance. *Cell host & microbe*. 2015;17(1):98-106.
65. Li Z, Huan C, Wang H, et al. TRIM21-mediated proteasomal degradation of SAMHD1 regulates its antiviral activity. *EMBO reports*. 2020;21(1):e47528.
66. Rocha-Perugini V, Suárez H, Álvarez S, et al. CD81 association with SAMHD1 enhances HIV-1 reverse transcription by increasing dNTP levels. *Nature microbiology*. 2017;2(11):1513-1522.

67. Zhang F, Sun J, Tang X, et al. Stabilization of SAMHD1 by NONO is crucial for Ara-C resistance in AML. *Cell death & disease*. 2022;13(7):590.
68. Liu J, Zhou T, Dong X, et al. De-ubiquitination of SAMHD1 by USP7 promotes DNA damage repair to overcome oncogenic stress and affect chemotherapy sensitivity. *Oncogene*. 2023;42(22):1843-1856.
69. Cardamone F, Iacovelli F, Chillemi G, Falconi M, Desideri A. A molecular dynamics simulation study decodes the early stage of the disassembly process abolishing the human SAMHD1 function. *Journal of computer-aided molecular design*. 2017;31(5):497-505.
70. Goldstone DC, Ennis-Adeniran V, Hedden JJ, et al. HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. *Nature*. 2011;480(7377):379-382.
71. Powell RD, Holland PJ, Hollis T, Perrino FW. Aicardi-Goutieres syndrome gene and HIV-1 restriction factor SAMHD1 is a dGTP-regulated deoxynucleotide triphosphohydrolase. *The Journal of biological chemistry*. 2011;286(51):43596-43600.
72. Amie SM, Bambara RA, Kim B. GTP is the primary activator of the anti-HIV restriction factor SAMHD1. *The Journal of biological chemistry*. 2013;288(35):25001-25006.
73. Morris ER, Caswell SJ, Kunzelmann S, et al. Crystal structures of SAMHD1 inhibitor complexes reveal the mechanism of water-mediated dNTP hydrolysis. *Nature communications*. 2020;11(1):3165.
74. Behrendt R, Schumann T, Gerbaulet A, et al. Mouse SAMHD1 has antiretroviral activity and suppresses a spontaneous cell-intrinsic antiviral response. *Cell reports*. 2013;4(4):689-696.
75. Baldauf HM, Pan X, Erikson E, et al. SAMHD1 restricts HIV-1 infection in resting CD4(+) T cells. *Nature medicine*. 2012;18(11):1682-1687.
76. Amie SM, Daly MB, Noble E, Schinazi RF, Bambara RA, Kim B. Anti-HIV host factor SAMHD1 regulates viral sensitivity to nucleoside reverse transcriptase inhibitors via modulation of cellular deoxyribonucleoside triphosphate (dNTP) levels. *The Journal of biological chemistry*. 2013;288(28):20683-20691.
77. Hollenbaugh JA, Tao S, Lenzi GM, et al. dNTP pool modulation dynamics by SAMHD1 protein in monocyte-derived macrophages. *Retrovirology*. 2014;11:63.
78. Kodigepalli KM, Li M, Bonifati S, et al. SAMHD1 inhibits epithelial cell transformation in vitro and affects leukemia development in xenograft mice. *Cell cycle (Georgetown, Tex)*. 2018;17(23):2564-2576.
79. Kodigepalli KM, Bonifati S, Tirumuru N, Wu L. SAMHD1 modulates in vitro proliferation of acute myeloid leukemia-derived THP-1 cells through the PI3K-Akt-p27 axis. *Cell cycle (Georgetown, Tex)*. 2018;17(9):1124-1137.
80. Herold N, Rudd SG, Ljungblad L, et al. Targeting SAMHD1 with the Vpx protein to improve cytarabine therapy for hematological malignancies. *Nature medicine*. 2017;23(2):256-263.
81. Tüngler V, Staroske W, Kind B, et al. Single-stranded nucleic acids promote SAMHD1 complex formation. *Journal of molecular medicine (Berlin, Germany)*. 2013;91(6):759-770.
82. Zhu C, Gao W, Zhao K, et al. Structural insight into dGTP-dependent activation of tetrameric SAMHD1 deoxynucleoside triphosphate triphosphohydrolase. *Nature communications*. 2013;4:2722.
83. Ji X, Wu Y, Yan J, et al. Mechanism of allosteric activation of SAMHD1 by dGTP. *Nature structural & molecular biology*. 2013;20(11):1304-1309.

84. Yan J, Kaur S, DeLucia M, et al. Tetramerization of SAMHD1 is required for biological activity and inhibition of HIV infection. *The Journal of biological chemistry*. 2013;288(15):10406-10417.
85. Thapa G, Bhattacharya A, Bhattacharya S. Dimeric Hold States of Anti-HIV Protein SAMHD1 are Redox Tunable. *Journal of chemical information and modeling*. 2020;60(12):6377-6391.
86. Brandariz-Nuñez A, Valle-Casuso JC, White TE, et al. Contribution of oligomerization to the anti-HIV-1 properties of SAMHD1. *Retrovirology*. 2013;10:131.
87. Ryoo J, Choi J, Oh C, et al. The ribonuclease activity of SAMHD1 is required for HIV-1 restriction. *Nature medicine*. 2014;20(8):936-941.
88. Hansen EC, Seamon KJ, Cravens SL, Stivers JT. GTP activator and dNTP substrates of HIV-1 restriction factor SAMHD1 generate a long-lived activated state. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;111(18):E1843-1851.
89. Miazzi C, Ferraro P, Pontarin G, Rampazzo C, Reichard P, Bianchi V. Allosteric regulation of the human and mouse deoxyribonucleotide triphosphohydrolase sterile α-motif/histidine-aspartate domain-containing protein 1 (SAMHD1). *The Journal of biological chemistry*. 2014;289(26):18339-18346.
90. Ji X, Tang C, Zhao Q, Wang W, Xiong Y. Structural basis of cellular dNTP regulation by SAMHD1. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;111(41):E4305-4314.
91. Koharudin LM, Wu Y, DeLucia M, Mehrens J, Gronenborn AM, Ahn J. Structural basis of allosteric activation of sterile α motif and histidine-aspartate domain-containing protein 1 (SAMHD1) by nucleoside triphosphates. *The Journal of biological chemistry*. 2014;289(47):32617-32627.
92. Jang S, Zhou X, Ahn J. Substrate Specificity of SAMHD1 Triphosphohydrolase Activity Is Controlled by Deoxyribonucleoside Triphosphates and Phosphorylation at Thr592. *Biochemistry*. 2016;55(39):5635-5646.
93. Wang Z, Bhattacharya A, Villacorta J, Diaz-Griffero F, Ivanov DN. Allosteric Activation of SAMHD1 Protein by Deoxynucleotide Triphosphate (dNTP)-dependent Tetramerization Requires dNTP Concentrations That Are Similar to dNTP Concentrations Observed in Cycling T Cells. *The Journal of biological chemistry*. 2016;291(41):21407-21413.
94. Arnold LH, Groom HC, Kunzelmann S, et al. Phospho-dependent Regulation of SAMHD1 Oligomerisation Couples Catalysis and Restriction. *PLoS pathogens*. 2015;11(10):e1005194.
95. Bhattacharya A, Wang Z, White T, et al. Effects of T592 phosphomimetic mutations on tetramer stability and dNTPase activity of SAMHD1 can not explain the retroviral restriction defect. *Scientific reports*. 2016;6:31353.
96. Franzolin E, Salata C, Bianchi V, Rampazzo C. The Deoxynucleoside Triphosphate Triphosphohydrolase Activity of SAMHD1 Protein Contributes to the Mitochondrial DNA Depletion Associated with Genetic Deficiency of Deoxyguanosine Kinase. *The Journal of biological chemistry*. 2015;290(43):25986-25996.
97. 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 reports*. 2020;31(6):107640.
98. Seamon KJ, Bumpus NN, Stivers JT. Single-Stranded Nucleic Acids Bind to the Tetramer Interface of SAMHD1 and Prevent Formation of the Catalytic Homotetramer. *Biochemistry*. 2016;55(44):6087-6099.

99. Seamon KJ, Sun Z, Shlyakhtenko LS, Lyubchenko YL, Stivers JT. SAMHD1 is a single-stranded nucleic acid binding protein with no active site-associated nuclease activity. *Nucleic acids research*. 2015;43(13):6486-6499.
100. Ryoo J, Hwang SY, Choi J, Oh C, Ahn K. SAMHD1, the Aicardi-Goutières syndrome gene and retroviral restriction factor, is a phosphorolytic ribonuclease rather than a hydrolytic ribonuclease. *Biochemical and biophysical research communications*. 2016;477(4):977-981.
101. Maharana S, Kretschmer S, Hunger S, et al. SAMHD1 controls innate immunity by regulating condensation of immunogenic self RNA. *Molecular cell*. 2022;82(19):3712-3728.e3710.
102. Orris B, Huynh KW, Ammirati M, et al. Phosphorylation of SAMHD1 Thr592 increases C-terminal domain dynamics, tetramer dissociation and ssDNA binding kinetics. *Nucleic acids research*. 2022;50(13):7545-7559.
103. Kretschmer S, Wolf C, König N, et al. SAMHD1 prevents autoimmunity by maintaining genome stability. *Annals of the rheumatic diseases*. 2015;74(3):e17.
104. Daddacha W, Koyen AE, Bastien AJ, et al. SAMHD1 Promotes DNA End Resection to Facilitate DNA Repair by Homologous Recombination. *Cell reports*. 2017;20(8):1921-1935.
105. Cabello-Lobato MJ, Wang S, Schmidt CK. SAMHD1 Sheds Moonlight on DNA Double-Strand Break Repair. *Trends in genetics : TIG*. 2017;33(12):895-897.
106. Kapoor-Vazirani P, Rath SK, Liu X, et al. SAMHD1 deacetylation by SIRT1 promotes DNA end resection by facilitating DNA binding at double-strand breaks. *Nature communications*. 2022;13(1):6707.
107. Coquel F, Silva MJ, Técher H, et al. SAMHD1 acts at stalled replication forks to prevent interferon induction. *Nature*. 2018;557(7703):57-61.
108. Akimova E, Gassner FJ, Schubert M, et al. SAMHD1 restrains aberrant nucleotide insertions at repair junctions generated by DNA end joining. *Nucleic acids research*. 2021;49(5):2598-2608.
109. Wang YT, Yuan B, Chen HD, et al. Acquired resistance of phosphatase and tensin homolog-deficient cells to poly(ADP-ribose) polymerase inhibitor and Ara-C mediated by 53BP1 loss and SAMHD1 overexpression. *Cancer science*. 2018;109(3):821-831.
110. Kodigepalli KM, Li M, Liu SL, Wu L. Exogenous expression of SAMHD1 inhibits proliferation and induces apoptosis in cutaneous T-cell lymphoma-derived HuT78 cells. *Cell cycle (Georgetown, Tex)*. 2017;16(2):179-188.
111. Wu Y, Niu Y, Wu Y, Chen X, Shen X, Gao W. SAMHD1 can suppress lung adenocarcinoma progression through the negative regulation of STING. *Journal of thoracic disease*. 2021;13(1):189-201.
112. St Gelais C, Kim SH, Maksimova VV, et al. A Cyclin-Binding Motif in Human SAMHD1 Is Required for Its HIV-1 Restriction, dNTPase Activity, Tetramer Formation, and Efficient Phosphorylation. *Journal of virology*. 2018;92(6).
113. de Silva S, Hoy H, Hake TS, Wong HK, Porcu P, Wu L. Promoter methylation regulates SAMHD1 gene expression in human CD4+ T cells. *The Journal of biological chemistry*. 2013;288(13):9284-9292.
114. Lee EJ, Seo JH, Park JH, et al. SAMHD1 acetylation enhances its deoxynucleotide triphosphohydrolase activity and promotes cancer cell proliferation. *Oncotarget*. 2017;8(40):68517-68529.
115. Mauney CH, Rogers LC, Harris RS, et al. The SAMHD1 dNTP Triphosphohydrolase Is Controlled by a Redox Switch. *Antioxidants & redox signaling*. 2017;27(16):1317-1331.

116. Wang Z, Bhattacharya A, White T, et al. Functionality of Redox-Active Cysteines Is Required for Restriction of Retroviral Replication by SAMHD1. *Cell reports*. 2018;24(4):815-823.
117. Simermeyer TL, Batalis S, Rogers LC, Zalesak OJ, Hollis T. Protein oxidation increases SAMHD1 binding ssDNA via its regulatory site. *Nucleic acids research*. 2023.
118. Martinat C, Cormier A, Tobaly-Tapiero J, et al. SUMOylation of SAMHD1 at Lysine 595 is required for HIV-1 restriction in non-cycling cells. *Nature communications*. 2021;12(1):4582.
119. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*. 2005;120(1):15-20.
120. Jin C, Peng X, Liu F, et al. MicroRNA-181 expression regulates specific post-transcriptional level of SAMHD1 expression in vitro. *Biochemical and biophysical research communications*. 2014;452(3):760-767.
121. Pilakka-Kanthikeel S, Raymond A, Atluri VS, et al. Sterile alpha motif and histidine/aspartic acid domain-containing protein 1 (SAMHD1)-facilitated HIV restriction in astrocytes is regulated by miRNA-181a. *Journal of neuroinflammation*. 2015;12:66.
122. Jin C, Peng X, Liu F, et al. Interferon-induced sterile alpha motif and histidine/aspartic acid domain-containing protein 1 expression in astrocytes and microglia is mediated by microRNA-181a. *AIDS (London, England)*. 2016;30(13):2053-2064.
123. Schindler C, Levy DE, Decker T. JAK-STAT signaling: from interferons to cytokines. *The Journal of biological chemistry*. 2007;282(28):20059-20063.
124. Kohnken R, Kodigepalli KM, Mishra A, Porcu P, Wu L. MicroRNA-181 contributes to downregulation of SAMHD1 expression in CD4+ T-cells derived from Sèzary syndrome patients. *Leukemia research*. 2017;52:58-66.
125. Riess M, Fuchs NV, Idica A, et al. Interferons Induce Expression of SAMHD1 in Monocytes through Down-regulation of miR-181a and miR-30a. *The Journal of biological chemistry*. 2017;292(1):264-277.
126. Nordlund P, Reichard P. Ribonucleotide reductases. *Annual review of biochemistry*. 2006;75:681-706.
127. Hofer A, Crona M, Logan DT, Sjöberg BM. DNA building blocks: keeping control of manufacture. *Critical reviews in biochemistry and molecular biology*. 2012;47(1):50-63.
128. Oh C, Ryoo J, Park K, et al. A central role for PI3K-AKT signaling pathway in linking SAMHD1-deficiency to the type I interferon signature. *Scientific reports*. 2018;8(1):84.
129. Gutiérrez-Chamorro L, Felip E, Bernat-Peguera A, et al. SAMHD1 expression modulates innate immune activation and correlates with ovarian cancer prognosis. *Frontiers in immunology*. 2023;14:1112761.
130. Li Y, Gao Y, Jiang X, et al. SAMHD1 silencing cooperates with radiotherapy to enhance anti-tumor immunity through IFI16-STING pathway in lung adenocarcinoma. *Journal of translational medicine*. 2022;20(1):628.
131. Chen Z, Jiang Z, Meng L, et al. SAMHD1, positively regulated by KLF4, suppresses the proliferation of gastric cancer cells through MAPK p38 signaling pathway. *Cell cycle (Georgetown, Tex)*. 2022;21(19):2065-2078.

132. Rogers JH, Gupta R, Reyes JM, et al. Modeling IKZF1 lesions in B-ALL reveals distinct chemosensitivity patterns and potential therapeutic vulnerabilities. *Blood advances*. 2021;5(19):3876-3890.
133. White TE, Brandariz-Nuñez A, Valle-Casuso JC, et al. The retroviral restriction ability of SAMHD1, but not its deoxynucleotide triphosphohydrolase activity, is regulated by phosphorylation. *Cell host & microbe*. 2013;13(4):441-451.
134. Cribier A, Descours B, Valadão AL, Laguette N, Benkirane M. Phosphorylation of SAMHD1 by cyclin A2/CDK1 regulates its restriction activity toward HIV-1. *Cell reports*. 2013;3(4):1036-1043.
135. Welbourn S, Dutta SM, Semmes OJ, Strebel K. Restriction of virus infection but not catalytic dNTPase activity is regulated by phosphorylation of SAMHD1. *Journal of virology*. 2013;87(21):11516-11524.
136. Batalis S, Rogers LC, Hemphill WO, Mauney CH, Ornelles DA, Hollis T. SAMHD1 Phosphorylation at T592 Regulates Cellular Localization and S-phase Progression. *Frontiers in molecular biosciences*. 2021;8:724870.
137. Tang C, Ji X, Wu L, Xiong Y. Impaired dNTPase activity of SAMHD1 by phosphomimetic mutation of Thr-592. *The Journal of biological chemistry*. 2015;290(44):26352-26359.
138. Bermejo M, López-Huertas MR, García-Pérez J, et al. Dasatinib inhibits HIV-1 replication through the interference of SAMHD1 phosphorylation in CD4+ T cells. *Biochemical pharmacology*. 2016;106:30-45.
139. Coiras M, Bermejo M, Descours B, et al. IL-7 Induces SAMHD1 Phosphorylation in CD4+ T Lymphocytes, Improving Early Steps of HIV-1 Life Cycle. *Cell reports*. 2016;14(9):2100-2107.
140. Bermejo M, Ambrosioni J, Bautista G, et al. Evaluation of resistance to HIV-1 infection ex vivo of PBMCs isolated from patients with chronic myeloid leukemia treated with different tyrosine kinase inhibitors. *Biochemical pharmacology*. 2018;156:248-264.
141. Manganaro L, Hong P, Hernandez MM, et al. IL-15 regulates susceptibility of CD4(+) T cells to HIV infection. *Proceedings of the National Academy of Sciences of the United States of America*. 2018;115(41):E9659-e9667.
142. White TE, Brandariz-Nuñez A, Han K, Sawyer SL, Kim B, Diaz-Griffero F. Modulation of LINE-1 Retrotransposition by a Human SAMHD1 Polymorphism. *Virology reports*. 2016;6:53-60.
143. Herrmann A, Wittmann S, Thomas D, et al. The SAMHD1-mediated block of LINE-1 retroelements is regulated by phosphorylation. *Mobile DNA*. 2018;9:11.
144. Gao W, Li G, Bian X, et al. Defective modulation of LINE-1 retrotransposition by cancer-associated SAMHD1 mutants. *Biochemical and biophysical research communications*. 2019;519(2):213-219.
145. Tramentozzi E, Ferraro P, Hossain M, Stillman B, Bianchi V, Pontarin G. The dNTP triphosphohydrolase activity of SAMHD1 persists during S-phase when the enzyme is phosphorylated at T592. *Cell cycle (Georgetown, Tex)*. 2018;17(9):1102-1114.
146. Schott K, Fuchs NV, Derua R, et al. Dephosphorylation of the HIV-1 restriction factor SAMHD1 is mediated by PP2A-B55α holoenzymes during mitotic exit. *Nature communications*. 2018;9(1):2227.
147. Blethrow JD, Glavy JS, Morgan DO, Shokat KM. Covalent capture of kinase-specific phosphopeptides reveals Cdk1-cyclin B substrates. *Proceedings of the National Academy of Sciences of the United States of America*. 2008;105(5):1442-1447.

148. St Gelais C, de Silva S, Hach JC, et al. Identification of cellular proteins interacting with the retroviral restriction factor SAMHD1. *Journal of virology*. 2014;88(10):5834-5844.
149. Yan J, Hao C, DeLucia M, et al. CyclinA2-Cyclin-dependent Kinase Regulates SAMHD1 Protein Phosphohydrolase Domain. *The Journal of biological chemistry*. 2015;290(21):13279-13292.
150. Ruiz A, Pauls E, Badia R, et al. Cyclin D3-dependent control of the dNTP pool and HIV-1 replication in human macrophages. *Cell cycle (Georgetown, Tex)*. 2015;14(11):1657-1665.
151. Hu J, Qiao M, Chen Y, et al. Cyclin E2-CDK2 mediates SAMHD1 phosphorylation to abrogate its restriction of HBV replication in hepatoma cells. *FEBS letters*. 2018;592(11):1893-1904.
152. Pauls E, Ruiz A, Badia R, et al. Cell cycle control and HIV-1 susceptibility are linked by CDK6-dependent CDK2 phosphorylation of SAMHD1 in myeloid and lymphoid cells. *Journal of immunology (Baltimore, Md : 1950)*. 2014;193(4):1988-1997.
153. Pauls E, Badia R, Torres-Torronteras J, et al. Palbociclib, a selective inhibitor of cyclin-dependent kinase4/6, blocks HIV-1 reverse transcription through the control of sterile α motif and HD domain-containing protein-1 (SAMHD1) activity. *AIDS (London, England)*. 2014;28(15):2213-2222.
154. Badia R, Angulo G, Riveira-Muñoz E, et al. Inhibition of herpes simplex virus type 1 by the CDK6 inhibitor PD-0332991 (palbociclib) through the control of SAMHD1. *The Journal of antimicrobial chemotherapy*. 2016;71(2):387-394.
155. Pauls E, Ruiz A, Riveira-Muñoz E, et al. p21 regulates the HIV-1 restriction factor SAMHD1. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;111(14):E1322-1324.
156. Valle-Casuso JC, Allouch A, David A, et al. p21 Restricts HIV-1 in Monocyte-Derived Dendritic Cells through the Reduction of Deoxynucleoside Triphosphate Biosynthesis and Regulation of SAMHD1 Antiviral Activity. *Journal of virology*. 2017;91(23).
157. Badia R, Pujantell M, Riveira-Muñoz E, et al. The G1/S Specific Cyclin D2 Is a Regulator of HIV-1 Restriction in Non-proliferating Cells. *PLoS pathogens*. 2016;12(8):e1005829.
158. St Gelais C, Kim SH, Ding L, et al. A Putative Cyclin-binding Motif in Human SAMHD1 Contributes to Protein Phosphorylation, Localization, and Stability. *The Journal of biological chemistry*. 2016;291(51):26332-26342.
159. Mlcochova P, Caswell SJ, Taylor IA, Towers GJ, Gupta RK. DNA damage induced by topoisomerase inhibitors activates SAMHD1 and blocks HIV-1 infection of macrophages. *The EMBO journal*. 2018;37(1):50-62.
160. Jáuregui P, Landau NR. DNA damage induces a SAMHD1-mediated block to the infection of macrophages by HIV-1. *Scientific reports*. 2018;8(1):4153.
161. Juliusson G, Antunovic P, Derolf A, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood*. 2009;113(18):4179-4187.
162. Zwaan CM, Kolb EA, Reinhardt D, et al. Collaborative Efforts Driving Progress in Pediatric Acute Myeloid Leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2015;33(27):2949-2962.
163. Lonetti A, Pession A, Masetti R. Targeted Therapies for Pediatric AML: Gaps and Perspective. *Frontiers in pediatrics*. 2019;7:463.
164. van der Velden VH, van der Sluijs-Geling A, Gibson BE, et al. Clinical significance of flowcytometric minimal residual disease detection in pediatric acute myeloid

leukemia patients treated according to the DCOG ANLL97/MRC AML12 protocol. *Leukemia*. 2010;24(9):1599-1606.
165. Edmund J, Thaliath LJ, Meleveedu K. Acute myeloid leukemia in the medically unfit elderly patients. *Leukemia research*. 2023;130:107306.
166. Tsesmetzis N, Paulin CBJ, Rudd SG, Herold N. Nucleobase and Nucleoside Analogues: Resistance and Re-Sensitisation at the Level of Pharmacokinetics, Pharmacodynamics and Metabolism. *Cancers*. 2018;10(7).
167. Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. *The New England journal of medicine*. 1994;331(14):896-903.
168. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.
169. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. *The New England journal of medicine*. 2017;377(5):454-464.
170. Rosenquist R, Bernard E, Erkers T, et al. Novel precision medicine approaches and treatment strategies in hematological malignancies. *Journal of internal medicine*. 2023.
171. Raetz EA, Teachey DT. T-cell acute lymphoblastic leukemia. *Hematology American Society of Hematology Education Program*. 2016;2016(1):580-588.
172. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. *Blood cancer journal*. 2017;7(6):e577.
173. Dombret H, Cluzeau T, Huguet F, Boissel N. Pediatric-like therapy for adults with ALL. *Current hematologic malignancy reports*. 2014;9(2):158-164.
174. Ronson A, Tvito A, Rowe JM. Treatment of Relapsed/Refractory Acute Lymphoblastic Leukemia in Adults. *Current oncology reports*. 2016;18(6):39.
175. Cohen A, Lee JW, Gelfand EW. Selective toxicity of deoxyguanosine and arabinosyl guanine for T-leukemic cells. *Blood*. 1983;61(4):660-666.
176. Kurtzberg J, Ernst TJ, Keating MJ, et al. Phase I study of 506U78 administered on a consecutive 5-day schedule in children and adults with refractory hematologic malignancies. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2005;23(15):3396-3403.
177. Kathpalia M, Mishra P, Bajpai R, Bhurani D, Agarwal N. Efficacy and safety of nelarabine in patients with relapsed or refractory T-cell acute lymphoblastic leukemia: a systematic review and meta-analysis. *Annals of hematology*. 2022;101(8):1655-1666.
178. Dunsmore KP, Winter SS, Devidas M, et al. Children's Oncology Group AALL0434: A Phase III Randomized Clinical Trial Testing Nelarabine in Newly Diagnosed T-Cell Acute Lymphoblastic Leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2020;38(28):3282-3293.
179. Franzolin E, Coletta S, Ferraro P, et al. SAMHD1-deficient fibroblasts from Aicardi-Goutières Syndrome patients can escape senescence and accumulate mutations. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2020;34(1):631-647.
180. Bowen NE, Temple J, Shepard C, et al. Structural and functional characterization explains loss of dNTPase activity of the cancer-specific R366C/H mutant SAMHD1 proteins. *The Journal of biological chemistry*. 2021;297(4):101170.

181. de Silva S, Wang F, Hake TS, Porcu P, Wong HK, Wu L. Downregulation of SAMHD1 expression correlates with promoter DNA methylation in Sézary syndrome patients. *The Journal of investigative dermatology*. 2014;134(2):562-565.
182. Merati M, Buethe DJ, Cooper KD, Honda KS, Wang H, Gerstenblith MR. Aggressive CD8(+) epidermotropic cutaneous T-cell lymphoma associated with homozygous mutation in SAMHD1. *JAAD case reports*. 2015;1(4):227-229.
183. Rassidakis GZ, Herold N, Myrberg IH, et al. Low-level expression of SAMHD1 in acute myeloid leukemia (AML) blasts correlates with improved outcome upon consolidation chemotherapy with high-dose cytarabine-based regimens. *Blood cancer journal*. 2018;8(11):98.
184. Jiang H, Li C, Liu Z, Shengjing H. Expression and Relationship of SAMHD1 with Other Apoptotic and Autophagic Genes in Acute Myeloid Leukemia Patients. *Acta haematologica*. 2020;143(1):51-59.
185. Zhu KW, Chen P, Zhang DY, et al. Association of genetic polymorphisms in genes involved in Ara-C and dNTP metabolism pathway with chemosensitivity and prognosis of adult acute myeloid leukemia (AML). *Journal of translational medicine*. 2018;16(1):90.
186. Marrero RJ, Cao X, Wu H, et al. SAMHD1 single nucleotide polymorphisms impact outcome in children with newly diagnosed acute myeloid leukemia. *Blood advances*. 2023;7(11):2538-2550.
187. Perelló-Reus CM, Català A, Caviedes-Cárdenas L, et al. FMS-like tyrosine kinase 3 (FLT3) modulates key enzymes of nucleotide metabolism implicated in cytarabine responsiveness in pediatric acute leukemia. *Pharmacological research*. 2020;151:104556.
188. Amin NA, Seymour E, Saiya-Cork K, Parkin B, Shedden K, Malek SN. A Quantitative Analysis of Subclonal and Clonal Gene Mutations before and after Therapy in Chronic Lymphocytic Leukemia. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2016;22(17):4525-4535.
189. Guièze R, Robbe P, Clifford R, et al. Presence of multiple recurrent mutations confers poor trial outcome of relapsed/refractory CLL. *Blood*. 2015;126(18):2110-2117.
190. Johansson P, Klein-Hitpass L, Choidas A, et al. SAMHD1 is recurrently mutated in T-cell prolymphocytic leukemia. *Blood cancer journal*. 2018;8(1):11.
191. Nadeu F, Martin-Garcia D, Clot G, et al. Genomic and epigenomic insights into the origin, pathogenesis, and clinical behavior of mantle cell lymphoma subtypes. *Blood*. 2020;136(12):1419-1432.
192. Wang T, Yue W, Tang G, et al. SAMHD1 Mutations and Expression in Mantle Cell Lymphoma Patients. *Frontiers in oncology*. 2021;11:763151.
193. Roider T, Wang X, Hüttl K, et al. The impact of SAMHD1 expression and mutation status in mantle cell lymphoma: An analysis of the MCL Younger and Elderly trial. *International journal of cancer*. 2021;148(1):150-160.
194. Bühler MM, Lu J, Scheinost S, et al. SAMHD1 mutations in mantle cell lymphoma are recurrent and confer in vitro resistance to nucleoside analogues. *Leukemia research*. 2021;107:106608.
195. Merrien M, Wasik AM, Ljung E, et al. Clinical and biological impact of SAMHD1 expression in mantle cell lymphoma. *Virchows Archiv : an international journal of pathology*. 2022;480(3):655-666.
196. Xagoraris I, Vassilakopoulos TP, Drakos E, et al. Expression of the novel tumour suppressor sterile alpha motif and HD domain-containing protein 1 is an

independent adverse prognostic factor in classical Hodgkin lymphoma. *British journal of haematology*. 2021;193(3):488-496.
197. Felip E, Gutiérrez-Chamorro L, Gómez M, et al. Modulation of DNA Damage Response by SAM and HD Domain Containing Deoxynucleoside Triphosphate Triphosphohydrolase (SAMHD1) Determines Prognosis and Treatment Efficacy in Different Solid Tumor Types. *Cancers*. 2022;14(3).
198. Rentoft M, Lindell K, Tran P, et al. Heterozygous colon cancer-associated mutations of SAMHD1 have functional significance. *Proceedings of the National Academy of Sciences of the United States of America*. 2016;113(17):4723-4728.
199. Yang CA, Huang HY, Chang YS, Lin CL, Lai IL, Chang JG. DNA-Sensing and Nuclease Gene Expressions as Markers for Colorectal Cancer Progression. *Oncology*. 2017;92(2):115-124.
200. Zhang Z, Li P, Sun P. Expression of SAMHD1 and its mutation on prognosis of colon cancer. *Oncology letters*. 2022;24(3):303.
201. You D, Zhang S, Yan S, et al. SAMHD1 as a prognostic and predictive biomarker in stage II colorectal cancer: A multicenter cohort study. *Frontiers in oncology*. 2022;12:939982.
202. Wang JL, Lu FZ, Shen XY, Wu Y, Zhao LT. SAMHD1 is down regulated in lung cancer by methylation and inhibits tumor cell proliferation. *Biochemical and biophysical research communications*. 2014;455(3-4):229-233.
203. Chen W, Cheng P, Jiang J, Ren Y, Wu D, Xue D. Epigenomic and genomic analysis of transcriptome modulation in skin cutaneous melanoma. *Aging*. 2020;12(13):12703-12725.
204. Daddacha W, Monroe D, Carver K, et al. Viral Particle-Mediated SAMHD1 Depletion Sensitizes Refractory Glioblastoma to DNA-Damaging Therapeutics by Impairing Homologous Recombination. *Cancers*. 2022;14(18).
205. Galmarini CM, Mackey JR, Dumontet C. Nucleoside analogues and nucleobases in cancer treatment. *The Lancet Oncology*. 2002;3(7):415-424.
206. King KM, Damaraju VL, Vickers MF, et al. A comparison of the transportability, and its role in cytotoxicity, of clofarabine, cladribine, and fludarabine by recombinant human nucleoside transporters produced in three model expression systems. *Molecular pharmacology*. 2006;69(1):346-353.
207. Baldwin SA, Yao SY, Hyde RJ, et al. Functional characterization of novel human and mouse equilibrative nucleoside transporters (hENT3 and mENT3) located in intracellular membranes. *The Journal of biological chemistry*. 2005;280(16):15880-15887.
208. Homminga I, Zwaan CM, Manz CY, et al. In vitro efficacy of forodesine and nelarabine (ara-G) in pediatric leukemia. *Blood*. 2011;118(8):2184-2190.
209. Parmar S, Seeringer A, Denich D, et al. Variability in transport and biotransformation of cytarabine is associated with its toxicity in peripheral blood mononuclear cells. *Pharmacogenomics*. 2011;12(4):503-514.
210. Arnold LH, Kunzelmann S, Webb MR, Taylor IA. A continuous enzyme-coupled assay for triphosphohydrolase activity of HIV-1 restriction factor SAMHD1. *Antimicrobial agents and chemotherapy*. 2015;59(1):186-192.
211. Seamon KJ, Stivers JT. A High-Throughput Enzyme-Coupled Assay for SAMHD1 dNTPase. *Journal of biomolecular screening*. 2015;20(6):801-809.
212. Yagüe-Capilla M, Rudd SG. A High-Throughput Enzyme-Coupled Activity Assay to Probe Small Molecule Interaction with the dNTPase SAMHD1. *Journal of visualized experiments : JoVE*. 2021(170).

213. Ballana E, Badia R, Terradas G, et al. SAMHD1 specifically affects the antiviral potency of thymidine analog HIV reverse transcriptase inhibitors. *Antimicrobial agents and chemotherapy*. 2014;58(8):4804-4813.
214. Ordonez P, Kunzelmann S, Groom HC, et al. SAMHD1 enhances nucleoside-analogue efficacy against HIV-1 in myeloid cells. *Scientific reports*. 2017;7:42824.
215. Knecht KM, Buzovetsky O, Schneider C, et al. The structural basis for cancer drug interactions with the catalytic and allosteric sites of SAMHD1. *Proceedings of the National Academy of Sciences of the United States of America*. 2018;115(43):E10022-e10031.
216. Herold N, Rudd SG, Sanjiv K, et al. SAMHD1 protects cancer cells from various nucleoside-based antimetabolites. *Cell cycle (Georgetown, Tex)*. 2017;16(11):1029-1038.
217. Schneider C, Oellerich T, Baldauf HM, et al. SAMHD1 is a biomarker for cytarabine response and a therapeutic target in acute myeloid leukemia. *Nature medicine*. 2017;23(2):250-255.
218. Oellerich T, Schneider C, Thomas D, et al. Selective inactivation of hypomethylating agents by SAMHD1 provides a rationale for therapeutic stratification in AML. *Nature communications*. 2019;10(1):3475.
219. Kicska GA, Long L, Hörig H, et al. Immucillin H, a powerful transition-state analog inhibitor of purine nucleoside phosphorylase, selectively inhibits human T lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;98(8):4593-4598.
220. Camici M, Garcia-Gil M, Pesi R, Allegrini S, Tozzi MG. Purine-Metabolising Enzymes and Apoptosis in Cancer. *Cancers*. 2019;11(9).
221. Buie LW, Epstein SS, Lindley CM. Nelarabine: a novel purine antimetabolite antineoplastic agent. *Clinical therapeutics*. 2007;29(9):1887-1899.
222. Rothenburger T, McLaughlin KM, Herold T, et al. SAMHD1 is a key regulator of the lineage-specific response of acute lymphoblastic leukaemias to nelarabine. *Communications biology*. 2020;3(1):324.
223. Rudd SG, Schaller T, Herold N. SAMHD1 is a barrier to antimetabolite-based cancer therapies. *Molecular & cellular oncology*. 2017;4(2):e1287554.
224. Ellison RR, Holland JF, Weil M, et al. Arabinosyl cytosine: a useful agent in the treatment of acute leukemia in adults. *Blood*. 1968;32(4):507-523.
225. Hollenbaugh JA, Shelton J, Tao S, et al. Substrates and Inhibitors of SAMHD1. *PloS one*. 2017;12(1):e0169052.
226. Herold N, Rudd SG, Sanjiv K, et al. With me or against me: Tumor suppressor and drug resistance activities of SAMHD1. *Experimental hematology*. 2017;52:32-39.
227. Castellví M, Felip E, Ezeonwumelu IJ, et al. Pharmacological Modulation of SAMHD1 Activity by CDK4/6 Inhibitors Improves Anticancer Therapy. *Cancers*. 2020;12(3).
228. Pauls E, Ballana E, Esté JA. Nucleotide embargo by SAMHD1: a strategy to block retroviral infection. *Antiviral research*. 2013;97(2):180-182.
229. Seamon KJ, Hansen EC, Kadina AP, et al. Small molecule inhibition of SAMHD1 dNTPase by tetramer destabilization. *Journal of the American Chemical Society*. 2014;136(28):9822-9825.
230. Rothenburger T, Thomas D, Schreiber Y, et al. Differences between intrinsic and acquired nucleoside analogue resistance in acute myeloid leukaemia cells. *Journal of experimental & clinical cancer research : CR*. 2021;40(1):317.
231. Mauney CH, Perrino FW, Hollis T. Identification of Inhibitors of the dNTP Triphosphohydrolase SAMHD1 Using a Novel and Direct High-Throughput Assay. *Biochemistry*. 2018;57(47):6624-6636.

232. Egleston M, Dong L, Howlader AH, et al. Deoxyguanosine-Linked Bifunctional Inhibitor of SAMHD1 dNTPase Activity and Nucleic Acid Binding. *ACS chemical biology*. 2023.
233. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674.
234. Yadav B, Wennerberg K, Aittokallio T, Tang J. Searching for Drug Synergy in Complex Dose-Response Landscapes Using an Interaction Potency Model. *Computational and structural biotechnology journal*. 2015;13:504-513.
235. Ghandi M, Huang FW, Jané-Valbuena J, et al. Next-generation characterization of the cancer cell line encyclopedia. *Nature*. 2019;569(7757):503-508.
236. Seashore-Ludlow B, Rees MG, Cheah JH, et al. Harnessing connectivity in a large-scale small-molecule sensitivity dataset. *Cancer discovery*. 2015;5(11):1210-1223.
237. Rees MG, Seashore-Ludlow B, Cheah JH, et al. Correlating chemical sensitivity and basal gene expression reveals mechanism of action. *Nature chemical biology*. 2016;12(2):109-116.
238. Team RC. R: A Language and Environment for Statistical Computing. 2022.
239. Wickham H, Chang W, Wickham MH. Package 'ggplot2'. *Create elegant data visualisations using the grammar of graphics Version*. 2016;2(1):1-189.
240. Martinez Molina D, Jafari R, Ignatushchenko M, et al. Monitoring drug target engagement in cells and tissues using the cellular thermal shift assay. *Science (New York, NY)*. 2013;341(6141):84-87.
241. Heinemann V, Xu YZ, Chubb S, et al. Inhibition of ribonucleotide reduction in CCRF-CEM cells by 2',2'-difluorodeoxycytidine. *Molecular pharmacology*. 1990;38(4):567-572.
242. Cerqueira NM, Fernandes PA, Ramos MJ. Understanding ribonucleotide reductase inactivation by gemcitabine. *Chemistry (Weinheim an der Bergstrasse, Germany)*. 2007;13(30):8507-8515.
243. Ruiz van Haperen VW, Veerman G, Vermorken JB, Peters GJ. 2',2'-Difluoro-deoxycytidine (gemcitabine) incorporation into RNA and DNA of tumour cell lines. *Biochemical pharmacology*. 1993;46(4):762-766.
244. Martinez Molina D, Nordlund P. The Cellular Thermal Shift Assay: A Novel Biophysical Assay for In Situ Drug Target Engagement and Mechanistic Biomarker Studies. *Annual review of pharmacology and toxicology*. 2016;56:141-161.
245. Rudd SG, Tsesmetzis N, Sanjiv K, et al. Ribonucleotide reductase inhibitors suppress SAMHD1 ara-CTPase activity enhancing cytarabine efficacy. *EMBO molecular medicine*. 2020;12(3):e10419.
246. Lonetti A, Cappellini A, Bertaina A, et al. Improving nelarabine efficacy in T cell acute lymphoblastic leukemia by targeting aberrant PI3K/AKT/mTOR signaling pathway. *Journal of hematology & oncology*. 2016;9(1):114.
247. Osborne WR, Spencer N. Partial purification and properties of the common inherited forms of adenosine deaminase from human erythrocytes. *The Biochemical journal*. 1973;133(1):117-123.
248. Shimony S, Liu Y, Valtis YK, et al. Nelarabine combination therapy for relapsed or refractory T-cell acute lymphoblastic lymphoma/leukemia. *Blood advances*. 2023;7(7):1092-1102.
249. Kuhlen M, Bleckmann K, Möricke A, et al. Neurotoxic side effects in children with refractory or relapsed T-cell malignancies treated with nelarabine based therapy. *British journal of haematology*. 2017;179(2):272-283.

250. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood*. 2022;140(12):1345-1377.
251. Juliusson G, Hagberg O, Lazarevic VL, et al. Improved survival of men 50 to 75 years old with acute myeloid leukemia over a 20-year period. *Blood*. 2019;134(18):1558-1561.
252. Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. *Blood reviews*. 2019;36:70-87.
253. Abrahamsson J, Forestier E, Heldrup J, et al. Response-guided induction therapy in pediatric acute myeloid leukemia with excellent remission rate. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2011;29(3):310-315.
254. Hoffman AE, Schoonmade LJ, Kaspers GJ. Pediatric relapsed acute myeloid leukemia: a systematic review. *Expert review of anticancer therapy*. 2021;21(1):45-52.