Towards targeting SAMHD1 to improve cytarabine therapy for hematological malignancies

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Acute myeloid leukemia (AML) is a hematological cancer where myeloid stem cells are transformed into malignant cells, filling and replacing the cells in a healthy bone marrow. Cytarabine is a nucleoside analog used in chemotherapy for hematological malignancies, such as AML, where it is combined with an anthracycline as a standard treatment. Nevertheless this nucleoside analog in itself is not an active cytotoxic compound. Cytarabine is a prodrug that needs metabolism to become its active triphosphate form, which disturbs DNA synthesis and therefore is effective against rapidly dividing cancer cells. However, despite treatment, this leukemia might relapse, when treatment responses are usually less effective. SAMHD1 (SAM and HD domain containing protein 1) has enzymatic activity that could explain why cytarabine treatment is not effective in some patient populations. SAMHD1 displays enzymatic activity against the active cytarabine metabolite, converting this active triphosphate (Ara-CTP) back to its inactive form (Ara-C), similarly to deoxynucleoside triphosphates (dNTPs) that are converted to their corresponding nucleoside core along with inorganic triphosphates (Figure 1).
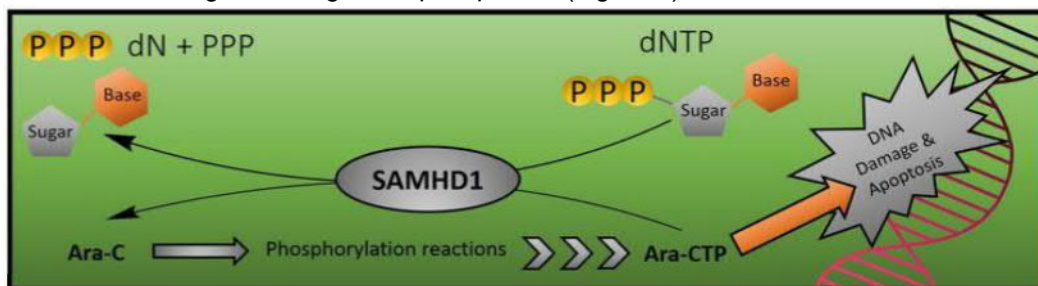

Figure 1: Both dNTPs and active cytarabine (Ara-CTP) are known substrates of SAMHD1. Ara-C is activated through phosphorylation reactions into the active triphosphate compound, ara-CTP that can incorporate into the genome, where it can cause DNA damage which leads to programmed cell death (e.g. apoptosis).

Recent studies indicate that inhibition of SAMHD1 could potentiate the efficacy of cytarabine treatment. SAMHD1 protein abundance has connections to ara-C response in leukemia cells (figure 2): ara-C cytotoxicity is enhanced in THP-1 SAMHD1 knock out (KO) cells in comparison to proficient cell line variant (THP-1 ctrl). In this thesis work, I optimized a cell-based drug-combination assay and used it to test and evaluate combination treatment of ara-C and candidate SAMHD1 inhibitor compounds in a panel of hematological cancer cell lines and indeed demonstrated that creating a SAMHD1 deficient phenotype with chemical compounds can potentiate cytarabine-induced cytotoxicity in SAMHD1 dependent manner.

Figure 2: Disruption of SAMHD1 can sensitize cells to cytarabine (ara-C). Ara-C dose-response curves for THP-1 ctrl (SAMHD1$^{+/+}$) and SAMHD1 KO (SAMHD1$^{-/-}$) cells after 96 hours incubation with ara-C. If SAMHD1 proficient cells are exposed to a SAMHD1 inhibitor, potentially we could see a better response (grey arrow) when SAMHD1 is not inactivating the cytotoxic ara-CTP.
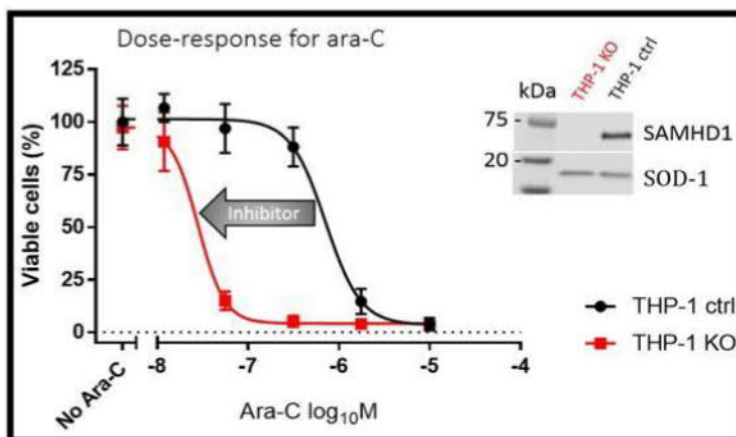
Keywords: Acute myeloid leukemia (AML), cytarabine (Ara-C), SAMHD1, synergy, combination treatment, precision medicine

Table of contents

1. Introduction

Globally, cancer is the second most common cause of disease related death. Annually, the incidence is increasing and new cancer cases are diagnosed more frequently. As an example, in the United States alone, approximately 20 000 new malignant cases are diagnosed each year (WHO Cancer Statistics). Cancer is a complex disease with a strong genetic background, as a series of mutations together with genomic instability is enough to transform a normal cell to a malignant one (Weinberg, 2014a). This tumor progression or tumorigenesis, might be a time consuming process and often cancer develops over time before diagnosis (Weinberg, 2014b). Nowadays, we can detect new cancer cases with improved screening methods and because of early detection, treatment has become more efficient (Weinberg, 2014c). However, relapses are nowadays the biggest challenge in cancer therapy. For example, cases where treatment fails because the tumor evolves and develops new mutations (Weinberg, 2014a). On the one hand, we can exploit cancer cells limitless replicative potential by manipulating nucleotide pools, the precursors of DNA and RNA, with nucleoside analogs, thus reducing malignancy (Weinberg, 2014d). On the other hand, new mutations can make the tumor even more resistant towards treatment (Weinberg, 2014e). It is crucial to consider genetic aspects before treatment, and for example, the genetic profile of the tumor could be used as a biomarker to direct treatment towards precision cancer medicine.

The malignancy related to my thesis is a hematological cancer, Acute Myeloid Leukemia (AML). During my thesis work, I examined drug combinations, which could be used to enhance the efficacy of current AML treatment. More precisely, could we potentiate the cytotoxic effect of current medication with another drug and hence reduce the amount of cytotoxic drug, simultaneously reducing its possible adverse-events?

In AML treatment, the nucleoside analog cytarabine (ara-C) combined with an anthracycline forms the backbone medication which is used to treat patients (Ossenkoppele and Bob, 2015). This induction medication has remained constant since 1973 but as stated previously the biggest challenge is a relapse, where malignancy returns and responses to treatment are weak, eventually

leading to poor prognosis (Saultz and Garzon, 2016; Kouchkovsky and Abdul-Hay, 2016).

Recently, SAM and HD domain containing protein 1 (SAMHD1) was identified as a possible therapeutic target, and its inhibition could benefit AML patients by enhancing current chemotherapy. SAMHD1 is an enzyme exhibiting phosphohydrolase activity towards deoxynucleoside triphosphates (dNTPs) (Goldstone et al., 2011). SAMHD1 converts nucleotides to their nucleoside core and releases an inorganic triphosphate (PPPi) in the same process. Also, the active metabolite of ara-C, ara-CTP, has been characterized as a substrate of SAMHD1 and this enzyme also protects leukemia cells from the cytotoxicity of ara-CTP *in vitro* (Herold et al., 2017a; Schneider et al., 2017). Additionally, in the same study Herold and co-workers observed that AML patients with higher SAMHD1 mRNA levels were not responding to ara-C treatment as well as patients with lower SAMHD1 mRNA levels.

This introduction begins with cancer – AML, particularly focusing on its current treatment. Then I will focus on the protein of interest SAMHD1 and especially, how it is connected to the current treatment. The third main topic will be drug synergy and finally, the research hypotheses and the objectives will be presented.

1.1 Acute Myeloid Leukemia

When myeloid stem cells in the bone marrow undergo malignant transformation they become cancer cells, more specifically hematological cancer cells. AML is a hematological cancer where immature cells begins to proliferate in an uncontrolled manner and finally these malignant myeloid cells can be observed in circulation (Saultz and Garzon, 2016). In normal, physiological conditions, these same cells are developing into red blood cells, platelets or leukocytes. When bone marrow is controlled by malignant cancer cells it cannot produce these healthy blood cells and this leads to the symptoms of AML. Patients are experiencing, for example: anemia, thrombocytopenia and additionally they have a higher infection risk because of the reduced number of functional neutrophils. (Kouchkovsky and Abdul-Hay, 2016).

AML is an acute disease, which means that it can progress rapidly to a severe stage, and, without treatment, it will be fatal in a few months. This hematological

cancer is most common amongst elderly people and in fact, it is the most common acute leukemia in adults (Kouchkovsky and Abdul-Hay, 2016). Even though we can treat AML and new treatments are developing, the long term survival of AML is quite poor. As an example, 5-year survival in elderly adults is less than 5%, and despite the treatment, approximately 70% of over 65-year patients are dying within one year after diagnosis (Meyers et al., 2013).

1.1.1 Current treatment

Even though 5-year survival is poor, there are still effective treatment options against AML. Current chemotherapy can be divided into induction and consolidation therapies, where induction therapy is aiming for remission of leukemia cells (Saultz and Garzon, 2016; Kouchkovsky and Abdul-Hay, 2016). However, AML has a high relapse rate and there is a need for more effective medications (Yang and Wang, 2018).

Compared to primary AML, relapsed AML is often more severe and difficult to treat (Saultz and Garzon, 2016; Yang and Wang, 2018). Treatment becomes even more complicated when circulating tumor cell cannot be targeted directly with surgical resection, which is usually used against solid primary tumors. However, more advanced treatment strategies are available, for instance, stem cell transplant (SCT) and antibody based treatments (Lamble et al., 2018). SCT is used to restore the functional bone marrow, which is achieved through the infusion of hematopoietic stem cells. These stem cells can be patient's own cells (autologous transplant) or cells from a donor (allogenic transplant) (Cornelissen and Blaise, 2018). One example of the antibody treatment is Gemtutsumab (Mylotarg®). Mylotarg is a specific drug-antibody conjugate, where an antibody is combined with a cytotoxic antineoplastic agent. Gemtutsumab is an antibody against myeloid cell surface antigen CD33 which is used to recognize hematopoietic cells (Schürch, 2018). However, this treatment is not completely specific towards malignant cells, leading to many side effects.

First line medication (the golden standard) in induction therapy is a combination of ara-C and an anthracycline (e.g. doxorubicin), a class of a chemotherapeutics which, for example, inhibits replication and promote cell death (Saultz and Garzon, 2016). Other chemotherapeutic agents are used during consolidation

therapy and, if necessary, SCT is considered (Kouchkovsky and Abdul-Hay, 2016). When cytotoxic drugs are used, the role of adverse events becomes more important, especially amongst elderly patients. As a part of cancer treatment, it is essential to evaluate risks and benefits of the treatment and consider other options, for example, whether we could potentiate current medication and hence avoid excessive toxicity caused by chemotherapeutic agents (e.g. ara-C).

1.1.2 Cytarabine

Ara-C is a classical, antineoplastic, chemotherapeutic agent used in the treatment of hematological malignancies. Itself, this nucleoside analog is not active (Figure 1), as it is a prodrug that needs metabolic reactions to be in its active triphosphate form (ara-CTP, Figure 2) (Shelton et al., 2016). The phosphorylation cascade of ara-C begins with the monophosphate (ara-CMP), continues to the diphosphate (ara-CDP) and finally ends with the active triphosphate (ara-CTP).
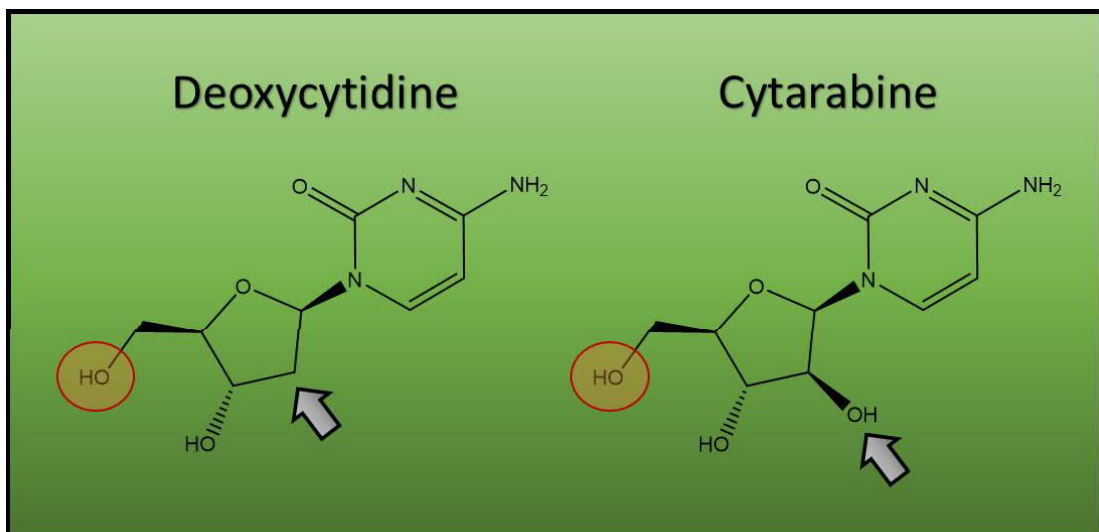
Figure 1, Deoxycytidine and nucleoside analog cytarabine. These two molecules are analogs and the only difference between is the additional hydroxyl group in cytarabine's furanose ring (arrow). The hydroxyl group (OH), important in phosphorylation, is marked inside the red circle.
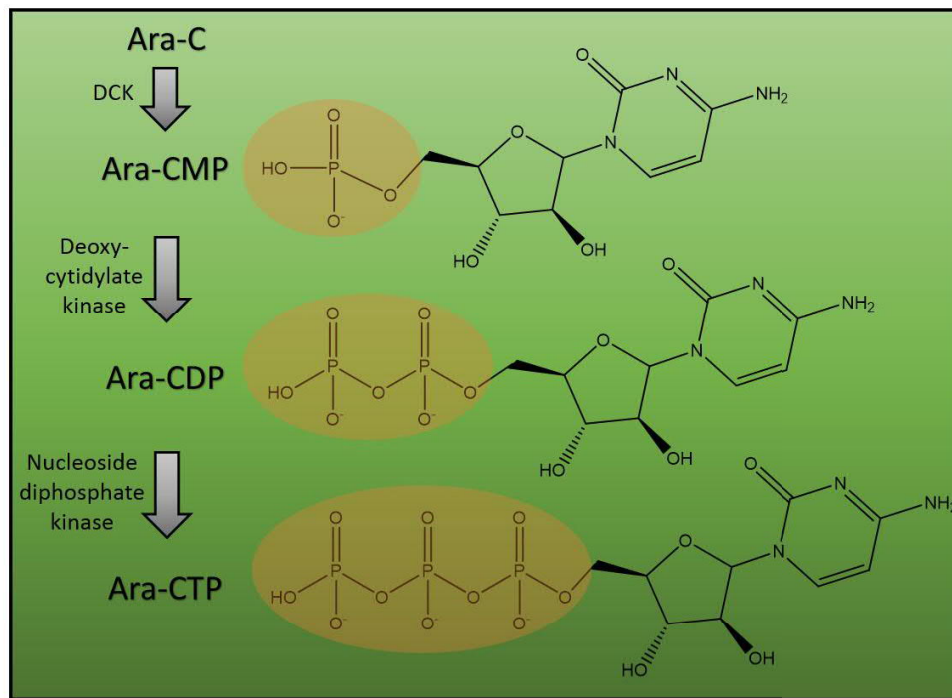
Figure 2, Different phosphorylation stages of ara-C. First phosphorylation product is monophosphate (CMP), second is diphosphate (CDP) and the last one is triphosphate (CTP). Enzymes involved in phosphorylation cascade are: Deoxycytidine kinase (DCK), deoxycytidylate kinase and nucleoside diphosphate kinase, respectively. The triphosphate, Ara-CTP, is biologically active form of ara-C.

Because ara-CTP mimics endogenous dCTP, it is a substrate of DNA polymerases, and so targets cells undergoing DNA synthesis, therefore ara-C is an effective drug against rapidly dividing cancer cells (Shelton et al., 2016). When this ara-C metabolite integrates into DNA, cells cannot efficiently copy their DNA and this leads to a stress response and eventually to programmed death (e.g. apoptosis or necroptosis). Cytarabine has been used to target rapidly dividing AML blasts since 1973 and it is still used in today's induction therapy. (Kouchkovsky and Abdul-Hay, 2016; Yang and Wang, 2018).

Unfortunately, some patient populations are not responding effectively to ara-C treatment (Yang and Wang, 2018). Cancer treatment is becoming more individualized and as a part of precision cancer medicine, new predictive markers are needed to evaluate patient risks and benefits from cytarabine therapy (Putten et al., 2011; Burnett et al., 2011).

1.2 SAMHD1

In 2009, SAMHD1 was first observed in human disease, in fact it was discovered that inherited, autosomal recessive, mutations of *SAMHD1* were the cause of

Aicardi-Goutières syndrome (AGS) (Rice et al., 2009). SAMHD1 is a homologue of murine interferon (IFN) γ-induced gene *Mg11* and has another role in Human Immunodeficiency Virus type 1 (HIV-1) infection as an anti-retroviral protein (Li et al., 2000). In 2011, it was discovered that this dNTP triphosphohydrolase acts as a restriction factor against HIV-1 in myeloid cells. In addition, it was observed that viral protein X (Vpx) can induce proteosomal degradation of SAMHD1 (Schwartz and Benkirane, 2011). This mechanism allows some viruses to avoid SAMHD1 mediated dNTP turnover and hence ensures the infection.

SAMHD1 has been implicated in the development of different malignancies for example in colon (Rentoft et al., 2016) and lung cancers (Wang et al., 2014). Furthermore, a function as a tumor suppressor has been suggested (Herold et al., 2017c; Clifford et al., 2014). The association to hematological malignancies has been disclosed for example in a whole exome sequencing study carried out in 2013 where they found that *SAMHD1* mutations could drive the development of chronic lymphocytic leukemia (CLL) and another study identified F545L mutation in early stages of CLL (Schuh et al., 2012; Landau et al., 2012).

1.2.1 Biological functions of SAMHD1

SAMHD1 is a dNTP triphosphohydrolase and this enzymatic activity is unique (in eukaryotes) and allosterically regulated, with substrates being all canonical dNTPs (i.e. dGTP, dATP, dTTP, dCTP) (Goldstone et al., 2011). Each SAMHD1 monomer contains two allosteric binding sites for nucleotides, these binding sites are regulating the formation of the catalytically active tetramer (Koharudin et al., 2014; Li et al., 2015). Once activated, SAMHD1 converts dNTPs into deoxynucleosides (dN) and inorganic triphosphates (PPPi).

SAMHD1 is a key enzyme in DNA precursor regulation. During different cell cycle phases, its enzymatic activity has been suggested to change to maintain DNA precursor pools (Franzolin et al., 2013). This cell cycle related regulation protects the cell and allows proliferation. For instance, lower enzyme activity during S-phase is necessary when dNTPs are needed for DNA replication, furthermore, lowering dNTP pools can protect the cell in virus infection. (Franzolin et al., 2013).

1.2.2 SAMHD1 and Cytarabine

As previously stated, SAMHD1 is responsible for reducing cytotoxicity of ara-C by converting the active ara-C metabolite, ara-CTP, back to its inactive state (Figure 3) (Herold et al., 2017a; Schneider et al., 2017). To clarify the interaction: ara-C is a substrate, but not an allosteric activator, of SAMHD1 (Herold et al., 2017a). Considering AML patient prognosis, this drug-enzyme interaction could play an important role in ara-C therapy. For instance, low ara-CTP levels (partly explained by SAMHD1 activity) might explain why responses to cytarabine treatment are not effective in some patients. Interestingly, there is a correlation between this enzyme expression and patient's treatment response: high SAMHD1 mRNA expression correlated with poor treatment response and additionally, SAMHD1 protein abundance can predict responses in cancer cell lines (Herold et al., 2017a; Schneider et al., 2017). So SAMHD1 has been identified as a risk factor for cytarabine therapy in AML and potentially the activity of this hydrolase could predict the efficacy of the treatment in AML patients.
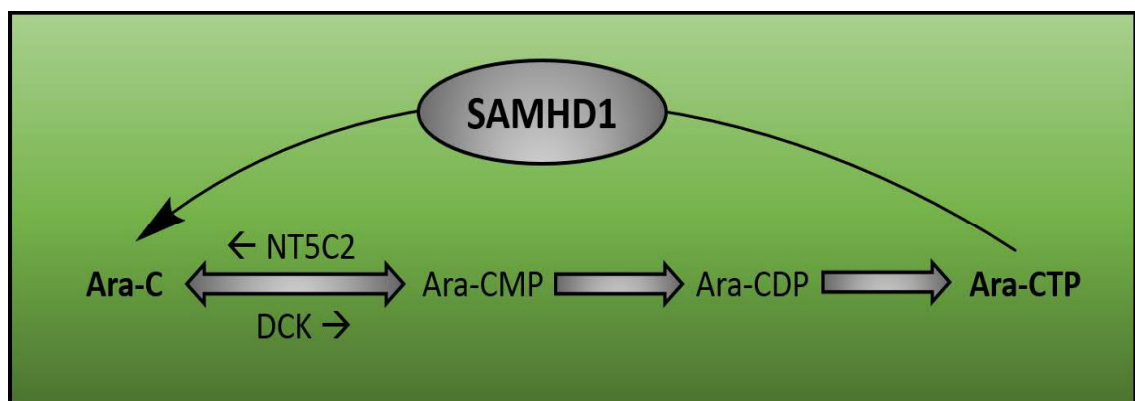
Figure 3, SAMHD1 converts biologically active ara-CTP back to inactive ara-C. Deoxycytidine kinase (DCK) is the rate-limiting enzyme in ara-C phosphorylation. Ara-CMP can be dephosphorylated by cytosolic 5'-nucleotidase-II (NT5C2). Additional deactivation mechanisms also exist: ara-C and ara-CMP can be deaminated to ara-U and ara-UMP, respectively (not showed in this figure).

Previously mentioned viral protein x (Vpx) can promote degradation of SAMHD1 and this information has been used to study the importance of SAMHD1 function in ara-C therapy. As an example, when SAMHD1 expression was reduced by Vpx treatment, patient-derived AML blasts were more sensitive to ara-C treatment (Herold et al., 2017a). Additionally, disruption of the gene itself can potentiate ara-C cytotoxicity. SAMHD1 knock out (KO) led to increased ara-CTP levels and, as a result of SAMHD1 disruption, ara-C treatment induced more DNA damage

and apoptosis. In addition, SAMHD1 KO AML mouse models were responding significantly better to ara-C treatment (Herold et al., 2017a).

In addition to effect on ara-C therapy, SAMHD1 has also been reported to reduce the efficacy of other antimetabolite-based therapies (Herold et al., 2017b). Any (2'S)2' -substituted nucleotides have been suggested to fit into the catalytic site of the enzyme and this includes several anticancer and antiviral nucleosides (Hollenbaugh et al., 2017). Therefore, targeting this enzyme could improve other antimetabolite-based therapies.

1.3 Drug synergy

Synergy in this context means a positive relationship between two individual agents, whose total effect is greater than the sum of individual drug effects. In my thesis project, I want to show that, by combining two drugs (ara-C and SAMHD1 inhibitor), we can see a stronger effect than using a single agent and for this purpose drug combination analyses are needed (Foucquier and Guedj, 2015).

As previously stated, SAMHD1 is limiting ara-C cytotoxicity, therefore we aimed to display a positive relationship between drug combination of candidate SAMHD1 inhibitor compounds and ara-C. To study these drug-drug interactions three reference models were used: zero interaction potency (ZIP), highest single agent (HSA) and bliss independence model. Data was generated using a dose-response matrix format and the relative cell viability measurements were analyzed with different synergy models.

Each synergy model is based upon a different assumption: HSA model compares the effects of combination treatment and the highest monotherapy, Bliss model assumes that two drugs are not interacting, instead they are acting independently on the phenotype, and ZIP model is combining Bliss and Loewe models and assumes that two drugs do not potentiate each other (Yadav et al., 2015).

ZIP was introduced in 2015 and this novel reference model combines the advantages of Bliss and Loewe models. ZIP method is based on delta scores in a response surface model and it compares the differences between individual drugs and their combinations. This comparison is based on the changes between the shapes of dose-response curves as well as the changes between potency in order to describe the drug interaction relationship in 3D matrix (Yadav et al., 2015).

1.4 Research Question, Hypothesis and Goals

As a result of several high throughput screens (HTS) conducted in the laboratory of Professor Thomas Helleday, several candidate small molecule inhibitors of SAMHD1 have been identified (unpublished). In this thesis work, my objectives were to optimize a cell-based assay where drug combinations of ara-C and candidate inhibitors could be tested and their possible therapeutic effects evaluated.

I started with single dose-response studies with ara-C and candidate inhibitors alone, using different cancer cell lines. Altogether 11 different hematological cancer cell lines were used in both single drug and drug combination (synergy) experiments. The hypotheses were that putative SAMHD1 inhibitors should sensitize cells to ara-C and if this sensitization is SAMHD1 dependent, then drug synergy of ara-C and candidate SAMHD1 inhibitors, should positively correlate with SAMHD1 protein abundance.

2. Results

2.1 Hypothesis

Our first hypothesis was that putative SAMHD1 inhibitors should sensitize leukemia cells to ara-C and this sensitization should be SAMHD1 dependent. We further hypothesized that, if ara-C potentiation is SAMHD1 dependent, then SAMHD1 protein expression should positively correlate with the synergy of a combination treatment of ara-C and a putative SAMHD1 inhibitor.

2.2 Hypothesis validation

The first experiments aimed to validate the research hypotheses. To begin with, we examined the role of SAMHD1 towards potentiation of ara-C cytotoxicity using different hematological cancer cells, including SAMHD1 proficient ($SAMHD1^{+/+}$) and deficient ($SAMHD1^{-/-}$) cell lines. Secondly, a drug-drug interactions between ara-C and putative SAMHD1 inhibitors were evaluated using drug synergy analysis.

2.2.1 SAMHD1 expression affects cytarabine response in leukemia cells

The relevance of SAMHD1 expression to ara-C cytotoxicity was examined in dose-response experiments using three different hematological cancer cell lines: THP-1, HuT-78 and HL-60, both SAMHD1 proficient ($SAMHD1^{+/+}$) and deficient

($SAMHD1^{-/-}$) variants, generated by our collaborator using the CRISPR/Cas9 gene editing technique. Additionally, rescue cell lines made from THP-1 $SAMHD1^{-/-}$ cells were used, either with restored wild-type (WT) SAMHD1 or expressing an inactive H233A SAMHD1 mutant. Western blot analysis of lysates from these cell lines revealed differences in SAMHD1 protein expression: THP-1 cells showing the highest expression, HuT-78 intermediate and HL-60 cells low SAMHD1 expression (Figure 6a). Additionally, as expected, no SAMHD1 was detected in the knockout (KO) variant and THP-1 rescue cell lines were both expressing SAMHD1 (Figure 6a).

The effect of ara-C upon cell proliferation after a 96 hour incubation was compared using the half maximal inhibitory concentration (IC_{50}) values, shown in Figure 6b-d. In general THP-1 cells, with higher SAMHD1 expression, had a weaker therapeutic response to ara-C than HuT-78 and HL-60 cell lines, which have lower SAMHD1 expression. Following incubation with ara-C, THP-1 KO and H233A (inactive SAMHD1) cells showed pronounced proliferation inhibition, whereas ctrl and WT cell lines did not respond as much to ara-C toxicity: IC_{50} for ctrl and WT was approximately 983 nM and for KO and H233A approximately 34 nM (Figure 6b). HuT-78 cells showed more cytotoxicity with KO cells (IC_{50} = 27.2 nM), in comparison to ctrl cells (IC_{50} = 362 nM) (Figure 6c). A slight difference in ara-C response was observed when HL-60 SAMHD1 ctrl and KO cells were incubated with ara-C, as IC_{50} values were respectively 30.9 nM and 8.5 nM (Figure 6d).
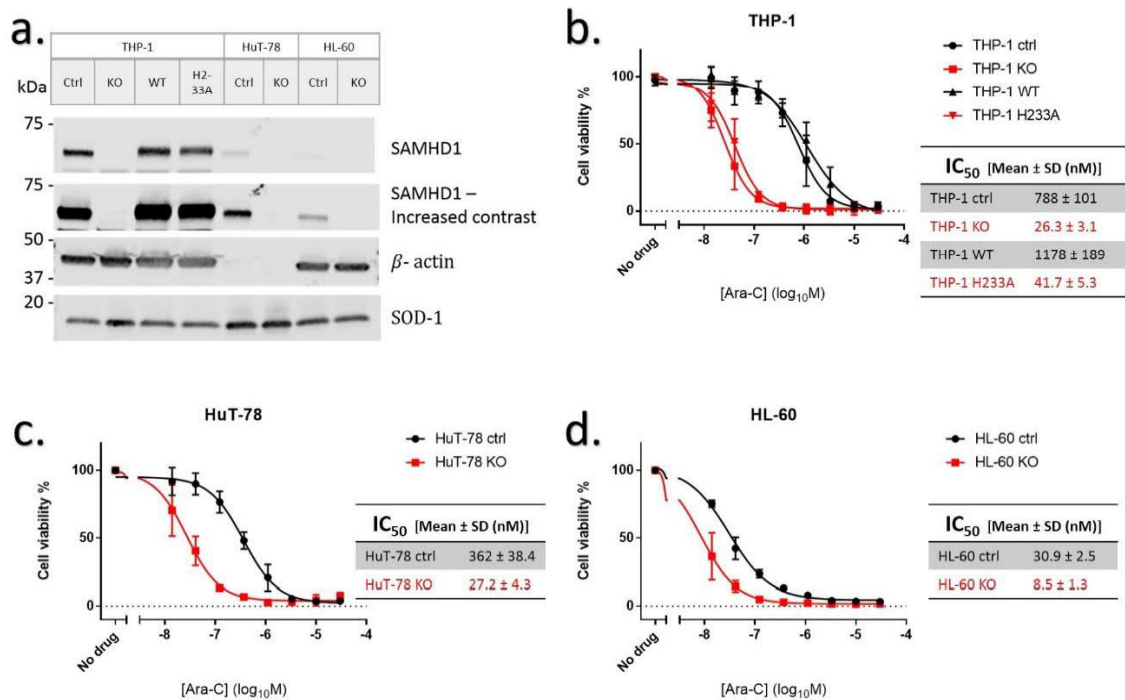
Figure 6, Ablation of SAMHD1 sensitizes hematological cancer cells to ara-C. **a**, Western blot analysis of THP-1 (ctrl, SAMHD1 KO, WT and H233A), HuT-78 (ctrl, SAMHD1 KO) and HL-60 (ctrl, SAMHD1 KO) cells. Representative of 2 independent experiments shown. **b-d**, Cell viability measured by resazurin assay after 96 hours incubation with ara-C of: **b**, THP-1 ctrl, SAMHD1 KO, WT, H233A; **c**, HuT-78 ctrl and SAMHD1 KO; **d**, HL-60 ctrl and SAMHD1 KO cells. Half maximal inhibitory concentrations for ara-C (IC_{50}) are indicated in nM. Mean values ± SD of 2 independent experiments are shown, both performed in triplicate. KO, SAMHD1 Knock Out; WT restored SAMHD1 wild-type expression; H233A, restored SAMHD1 expression of an inactive mutant.

Taken together, these data show that cell lines with higher SAMHD1 expression are more resistant to ara-C, whereas lower ara-C IC_{50} values were observed in cell lines with low SAMHD1 expression. Indeed, SAMHD1 expression affects ara-C response, more specifically it limits the cytotoxicity of ara-C in examined cell lines. Results were similar than in already published studies (Herold et al., 2017; Schneider et al., 2017), and so we continued investigating the potential role of chemical inhibition of SAMHD1 in potentiating ara-C cytotoxicity *in vitro*.

2.2.2 Cytotoxicity of cytarabine can be potentiated with putative SAMHD1 inhibitors

SAMHD1 limits the cytotoxicity of ara-C (Figure 6). To further validate our hypothesis, we wanted to examine whether the cytotoxicity of ara-C could be potentiated by chemically inhibiting SAMHD1. We began to examine drug-drug interactions, such as synergy, in drug combinations of ara-C and candidate SAMHD1 inhibitors using THP-1 ctrl and SAMHD1 KO cells. As THP-1 cells express the most SAMHD1 protein and KO of SAMHD1 resulted in the greatest sensitization to ara-C (amongst the examined cell lines, Figure 6a), we expected that this choice of cell line would be optimal as a model system for subsequent studies. Relative cell viability values following 96 hours incubation with drug combinations (ara-C + candidate inhibitor A or B) were used to evaluate the drug-drug interaction with the online tool synergyfinder, developed at the Institute for Molecular Medicine Finland (FIMM) (Yadav et al., 2015).

Incubation of cells with both drug combinations (ara-C + inhibitor A or B) led to a potentiation of ara-C cytotoxicity (Figure 7). In dose-response curves, the more inhibitor was used the less ara-C was needed to inhibit cell proliferation. Importantly, specificity to SAMHD1 was demonstrated as the shift in ara-C IC_{50} was observed in the THP-1 ctrl cell line but not with SAMHD1 KO cells (Figure 7a). The effects of drug combinations upon cell viability were visualized as heatmaps, where results were in line with the original dose-response curves. This allowed us to visualize how THP-1 ctrl cells' sensitivity to ara-C was enhanced by the candidate SAMHD1 inhibitors, however, similar potentiation was not observed with SAMHD1 KO cells (Figure 7b). The drug combination was evaluated with 3 different synergy models (ZIP, HSA, and Bliss), and with all models, drug combinations were beneficial in comparison to the effect of drugs independently (either ara-C, inhibitor compound A or compound B alone). In THP-1 ctrl cells, ara-C combined with inhibitor A produced an average of 7% enhanced proliferation inhibition over the expected additive result in the synergy landscape evaluated by the ZIP model (Figure 7c). Moreover, with optimal concentrations, this combination produced almost 40% more proliferation inhibition in comparison to the expected single drug responses (Figure 7c). KO cells were relatively less resistant to ara-C as ctrl cells and produced a weaker synergistic effect (with an average delta score of 4%) (Figure 7c).
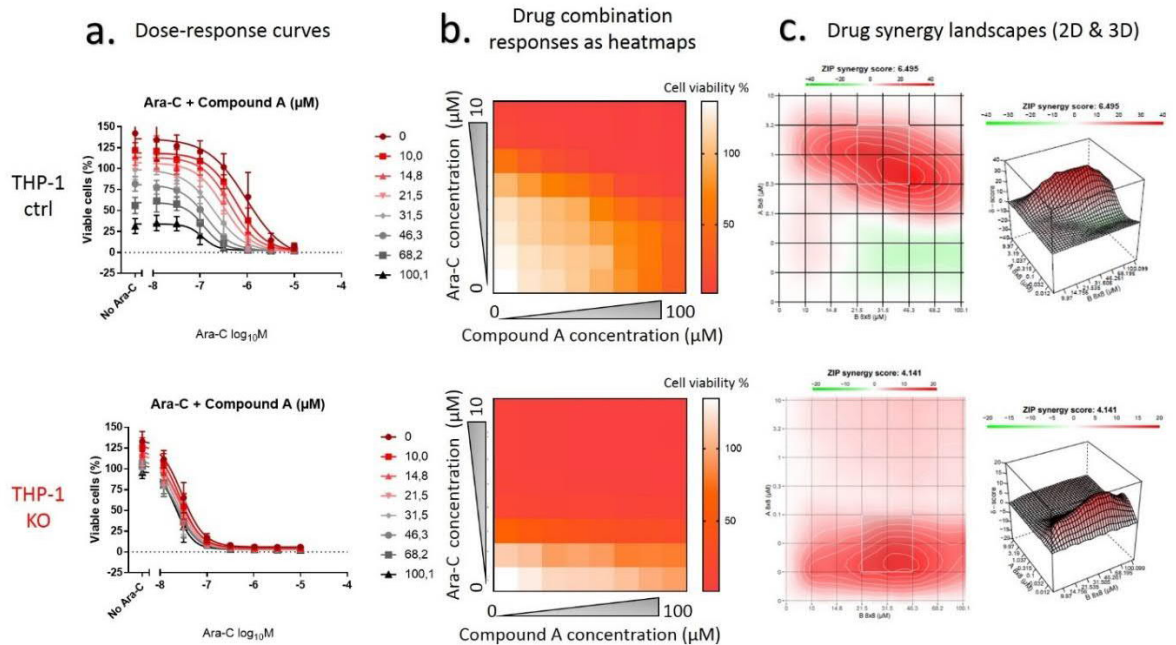
Figure 7, A drug combination of potential SAMHD1 inhibitor (compound A) and ara-C is synergistic and enhances the ara-C cytotoxicity in THP-1 cells in a SAMHD1 dependent manner. a, Proliferation inhibition analysis of combination treatment ara-C + compound A in THP-1 SAMHD1 proficient (ctrl) and deficient (KO) cells. Viability was measured using resazurin assay after 96 hours drug incubation. **b,** Relative viability from dose-responses was analyzed with FIMM's online tool synergyfinder, and results are presented with inhibition heatmaps and synergy landscapes. Drug combination responses as heatmaps for combination ara-C and compound A in THP-1 SAMHD1 proficient (ctrl) and deficient (KO) cells. Grey triangles indicate concentration (µM) of ara-C (y-axis) and compound A (x-axis) in a coordinate system where the origin is equal to zero. **c**, Synergy landscapes (2D & 3D) for THP-1 SAMHD1 proficient (ctrl) and deficient (KO) cells after 96 hours incubation with drug combination of ara-C (y-axis) and compound A (x-axis). An average synergy score (delta-score) of 0 was considered as additive result, less than -5 as antagonistic and over 5 as synergistic (delta score for KO cells was 4% and for ctrl cells 7%). The most synergistic area is annotated with a pale square.

Combination of ara-C and inhibitor B was also synergistic in proliferation inhibition, and with tested drug concentrations an average of 5% more inhibition was observed in THP-1 ctrl cells, over the expected effects of an individual drugs (Appendix 1). Similarly, with optimal concentrations, this drug combination enhanced the inhibition almost 40% in comparison to additive drug responses. Again, compound B and ara-C showed a weak synergistic effect with THP-1 SAMHD1 KO cells, additionally, inhibitor affected ara-C response with ctrl cells but not with KO cells (Appendix 1).

Taken together, both drug combinations showed a positive, synergistic, effect in THP-1 ctrl cells and a weaker synergistic response in SAMHD1 KO variant, consistent with compound A and B inhibiting SAMHD1. In conclusion, the cytotoxicity of ara-C can be potentiated by a small molecule inhibitor of SAMHD1, thereby creating a phenotype mimicking SAMHD1 deficiency. The hypothesis was validated and to further investigate the therapeutic potential of the drug combinations, I began to develop a cell-based assay suitable for high throughput screening. Aiming for testing candidate inhibitors and ara-C responses in a panel of different hematological cell lines.

2.3 Developing a cell-based assay for synergy experiments

One aim of this thesis is to develop a cell-based assay that can be used to evaluate SAMHD1 inhibitor candidates and to compare the effects of drug combinations in different hematological cancer cell lines. Dose-response curves have a crucial role in drug-drug interaction evaluation: prior to evaluating drug synergy, the optimal concentrations of each drug were determined to ensure complete dose-response data. In addition, drug matrix used in synergy experiment was optimized aiming to increase a throughput of the assay, similarly reducing required resources (e.g. reagents).

2.3.1 Individual dose-responses in a panel of cancer cell lines

Before testing drug combination effects in a panel of hematological cancer cell lines, I investigated individual dose-responses with single compounds to determine the optimal concentrations for subsequent synergy experiments. The aim was to ascertain complete dose-response curves (viability range from 0% to 100%) with all cell lines for each compound (compounds A, B, and ara-C). Cell lines used in this thesis are listed in Table 1.

Table 1, Hematological cell lines used in this thesis.

Cell line	Description
THP-1	Acute monocytic leukemia, patient derived human monocytic cell line
HuT-78	Sezary Syndrome (cutaneous T-cell lymphoma)
HL-60	Acute promyelocytic leukemia
KBM-7	Chronic myelogenous leukemia (CML)
K562	Chronic myelogenous leukemia
CCRF-CEM	Acute lymphoblastic leukemia
MV-4-11	Biphenotypic B -myelomonocytic leukemia
NB-4	Acute promyelocytic leukemia
Jurkat	Acute T-cell leukemia
MOLT-16	T-cell acute lymphoblastic leukemia (T-ALL)
MOLT-4	Acute lymphoblastic leukemia
LCL-534	Immortalized human B-cell line

Whilst complete dose-response curves for compound B and ara-C could be obtained, the chosen concentrations of compound A could not produce a complete response, despite being relatively high (Figure 8). Since 0% viability was not reached with compound A (Figure 8b), another maximal concentration was decided and the experiment was repeated. All in all, the dose-response experiment was performed three times and drug concentrations were obtained for subsequent synergy experiments. The next step was to optimize the drug synergy matrix experiment suitable for screening and evaluating drug combination effects in hematological cancer cell lines.
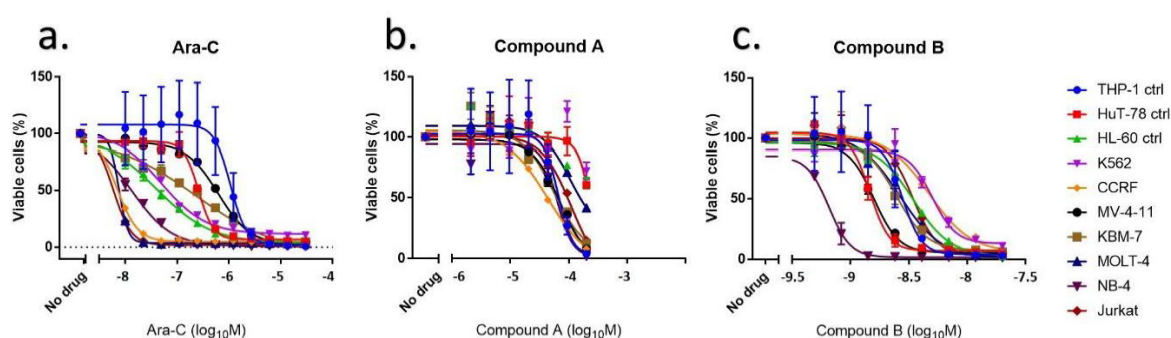

Figure 8, An example of individual dose-response experiments with different hematological cancer cell lines. Cell viability, measured by resazurin assay of different hematological cancer cell lines (cell line details in Table 1) after 96 hours incubation with: **a**, ara-C; **b**, compound A or **c**, compound B. Mean

values ± SD in a representative of 3 independent experiments, performed in duplicate.

2.3.2 Drug synergy matrix optimization with THP-1 cell line

According to previous results, THP-1 cells have the highest SAMHD1 expression amongst the hematological cancer cell lines studied (Figure 6a). Additionally, these cells provide a large difference in ara-C IC_{50} between SAMHD1-proficient (THP-1 ctrl) and deficient (THP-1 KO) variants (Figure 6b). Thus, to ensure maximal sensitivity of the assay we performed drug matrix optimization using THP-1 cell lines.

Altogether 4 different drug matrices were tested (8x8, 6x6, 5x5 and 4x4) and both candidate inhibitors (A and B) were independently combined with ara-C in these drug concentration matrices. Prior to synergy analysis, proliferation inhibition dose-response curves were analyzed (Figure 9). When inhibitor concentrations were increasing, toxicity to ara-C, after 96 hours incubation, were enhanced. With both inhibitors (A and B), the amount of ara-C needed to achieve the IC_{50} was reduced in THP-1 ctrl cells but not in KO cells. In THP-1 ctrl cell line, enhanced ara-C cytotoxicity was detected with all drug matrices: 8x8 and 6x6 (Figure 9a) as well as 5x5 and 4x4 (Figure 9c). However, THP-1 KO cell response to ara-C remained constant across tested drug matrices: 8x8 and 6x6 (Figure 9b) 5x5 and 4x4 (Figure 9d).
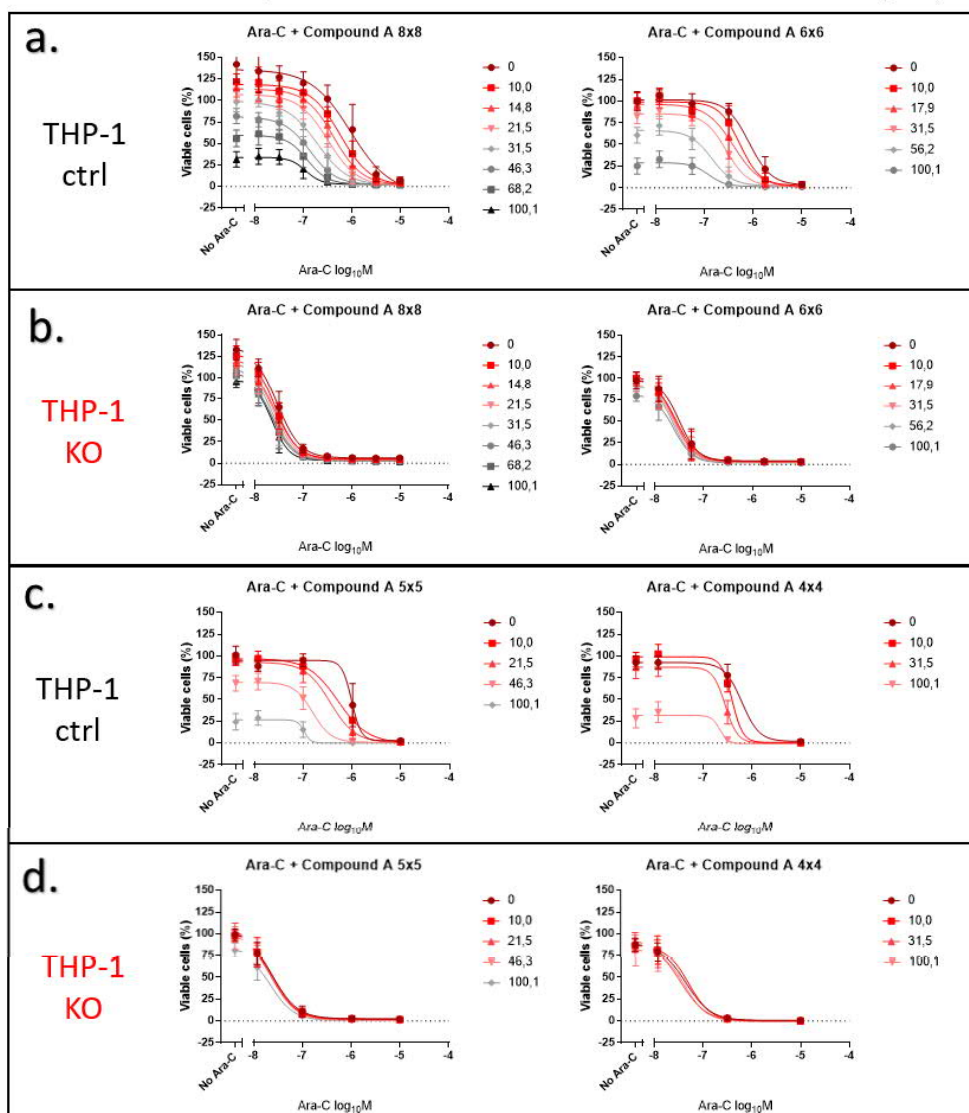
Figure 9, Combined ara-C dose-response curves from synergy optimization study with candidate SAMHD1 inhibitor A. Ara-C dose-response curves for THP-1 ctrl and SAMHD1 KO cells. Cell viability was determined using a resazurin assay of THP-1 ctrl (**a,c**) and SAMHD1 KO (**b,d**) cells, following 96 hours incubation with a combination of ara-C and compound A. Drug matrices (**a,b**) 8x8, 6x6; (**c,d**) 5x5 and 4x4, were compared. Compound A concentrations are indicated in µM. Mean values ± SD of 4 independent experiments, performed in duplicate (plate layout in Appendix 2).

Dose-responses for compound A and ara-C (Figure 9) as well as for compound B and ara-C (Appendix 3) showed a similar trend across all tested drug matrices (matrices with 4, 5, 6 or 8 concentration pairs). Based upon these results, the assay was working and even with smaller matrices (5x5 and 4x4), we observed potentiation of ara-C cytotoxicity with both candidate compounds A and B.

Relative viability values were used to assess drug synergy with FIMM's online tool synergyfinder. As we were optimizing this assay, increasing its throughput without compromising the quality of the data, the focus was on the differences between the drug matrices and synergy models. Comparison between used drug matrices and synergy models was performed with average synergy scores (delta-scores) obtained from synergy analysis with ZIP, HSA, and Bliss reference models.

When an average synergy score, delta score, above 5 was considered as synergistic, all used matrices produced a synergistic outcome with THP-1 ctrl cells, and a weaker synergistic effect with THP-1 SAMHD1 KO cells, according to ZIP synergy model (Figure 10).

Figure 10, Drug matrix optimization results from ZIP synergy model. Relative dose-response values from resazurin viability experiments (n = 4) of THP-1 ctrl and SAMHD1 KO cells after 96 hours incubation with drug combinations (ara-C and compound A or B), were used to obtain an average synergy scores (delta scores – y-axis) from FIMM's online tool synergyfinder. On the top part of the figure: average synergy scores for drug combination ara-C + compound A and at the lower part of the figure: combination ara-C + compound B. Individual data points indicate mean values from 4 independent experiments, each performed in duplicate, error bars indicate SD. THP-1 ctrl cells are represented in black and THP-1 SAMHD1 KO cells in red. Drug matrices were

compared with an average synergy scores (delta-scores) and delta equal to zero was considered as an additive response, above 5 as synergistic and less than -5 as antagonistic interaction, the grey rectangle is indicating the area between antagonistic and weak synergistic interaction (delta from -5 to 5). Statistical significances between drug matrices are presented in Table 2.

Consistent with the results obtained using the ZIP model (presented above in Figure 10), analysis with the HSA model showed that within all tested matrices a synergistic interaction was observed with both candidate inhibitors in THP-1 ctrl cells while a weaker synergistic interaction was observed in KO cells (Figure 11).
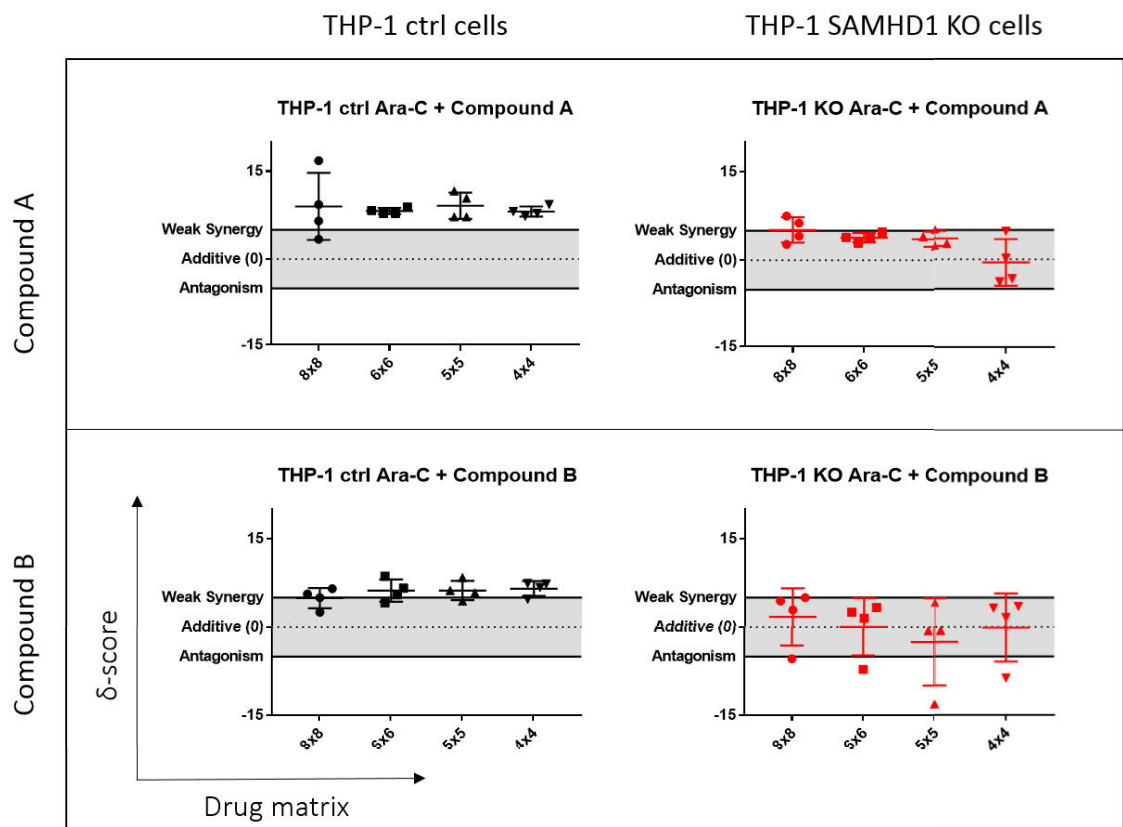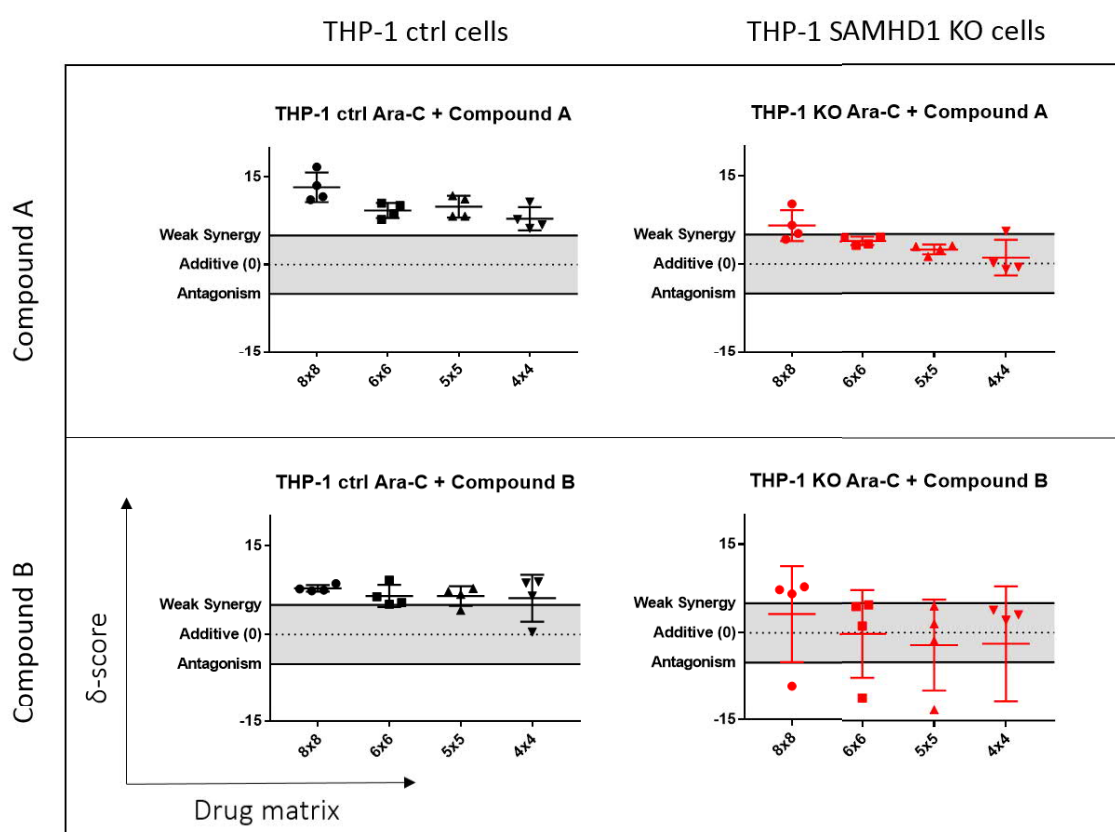

Figure 11, Drug matrix optimization results from HSA synergy model. Relative dose-response values from resazurin viability experiments (n = 4) of THP-1 ctrl and SAMHD1 KO cells after 96 hours incubation with drug combinations (ara-C and compound A or B), were used to obtain an average synergy scores (delta scores – y-axis) from FIMM's online tool synergyfinder. On the top part of the figure: average synergy scores for drug combination ara-C + compound A and at the lower part of the figure: combination ara-C + compound B. Individual data points indicate mean values from 4 independent experiments, each performed in duplicate, error bars indicate SD. THP-1 ctrl cells are represented in black and THP-1 SAMHD1 KO cells in red. Drug matrices were compared with an average synergy scores (delta-scores) and delta equal to zero was considered as an additive response, above 5 as a synergistic and less than -5 as an antagonistic interaction, the grey rectangle is indicating the area between

Differences between matrices were also assessed with the Bliss synergy model. When different matrices were compared, all of them showed similar synergistic interaction in THP-1 ctrl cells, whereas a weaker synergistic interaction was detected in KO cells (Appendix 4).

In conclusion, all tested synergy models (ZIP, HSA, and Bliss) were in line with each other and agreed upon synergistic or antagonistic interactions detected even with the smaller drug matrices (5x5 and 4x4). The mean values between drug matrices were compared with one-way analysis of variance (one-way ANOVA) (Table 2). Altogether 72 comparisons were analyzed and only 3 drug matrix pairs produced a statistically significant difference between their mean values. When synergy was evaluated with Bliss model: THP-1 ctrl cells exposed to drug combination of ara-C and compound B produced a statistically significant difference between mean values of drug matrices 8x8 and 5x5 (P = 0.0210), nevertheless the same comparison was not as significant when analyzed using ZIP and HSA models. However, HSA model produced 2 significant comparisons, both with THP-1 SAMHD1 KO cells: Drug combination of ara-C and compound A, mean values between drug matrices 6x6 and 5x5 (P = 0.0455) and a combination of ara-C and compound B between drug matrices 8x8 and 4x4 (P = 0.0306). Again, comparisons between these matrices were not showing statistically significant differences in analysis with ZIP and Bliss models (Table 2).

Table 2, Statistical significance between examined drug matrices. In drug synergy optimization results, there were 3 statistically significant drug matrix pairs, when differences between matrices were examined with one-way analysis of variance (one-way ANOVA), where P = <0.05 indicates statistical significance, bold values.

Cell line	Drug combination	Synergy model	Compared drug matrices (P - values)					
			8x8 *vs.* 6x6	8x8 *vs.* 5x5	8x8 *vs.* 4x4	6x6 *vs.* 5x5	6x6 *vs.* 4x4	5x5 *vs.* 4x4
THP-1 ctrl	Ara-C + Compound A	ZIP	0.9917	>0.9999	0.9906	0.9045	0.9970	0.9025
		HSA	0.2929	0.2327	0.1763	0.9705	0.5483	0.6277
		Bliss	0.9764	>0.9999	0.8854	0.8512	0.6695	0.5194
	Ara-C + Compound B	ZIP	0.2252	0.2083	0.3802	0.4112	0.9940	0.3675
		HSA	0.6493	0.4171	0.9312	>0.9999	0.9929	0.9905
		Bliss	0.4562	**0.0210**	0.9749	0.9981	0.9219	0.8330
THP-1 SAMHD1 KO	Ara-C + Compound A	ZIP	0.4999	0.5589	0.1988	0.9896	0.3465	0.1581
		HSA	0.3213	0.1155	0.1625	**0.0455**	0.4859	0.8046
		Bliss	0.4974	0.5396	0.2190	0.9903	0.3420	0.2427
	Ara-C + Compound B	ZIP	0.9917	>0.9999	0.9906	0.9045	0.9970	0.9025
		HSA	0.1180	0.1107	**0.0306**	0.5924	0.6518	0.9992
		Bliss	0.2735	0.1515	0.5032	0.6635	0.9807	0.9802

Taken together, all examined drug concentration matrices were valid to be used to detect and evaluate drug-drug interactions in subsequent experiments. However, smaller drug matrices would be more appropriate for high throughput screening and therefore, we compared drug matrices 5x5 and 4x4. Both drug matrices indicated similar synergistic interaction (Figures 10 and 11) but dose-response curves, with only 4 drug concentrations, were not as accurate and reliable as with 5 (Figure 12) and for this reason, matrix 5x5, was decided to be used as optimized drug matrix.

Ara-C dose-responses with candidate SAMHD1 inhibitors A and B (µM)
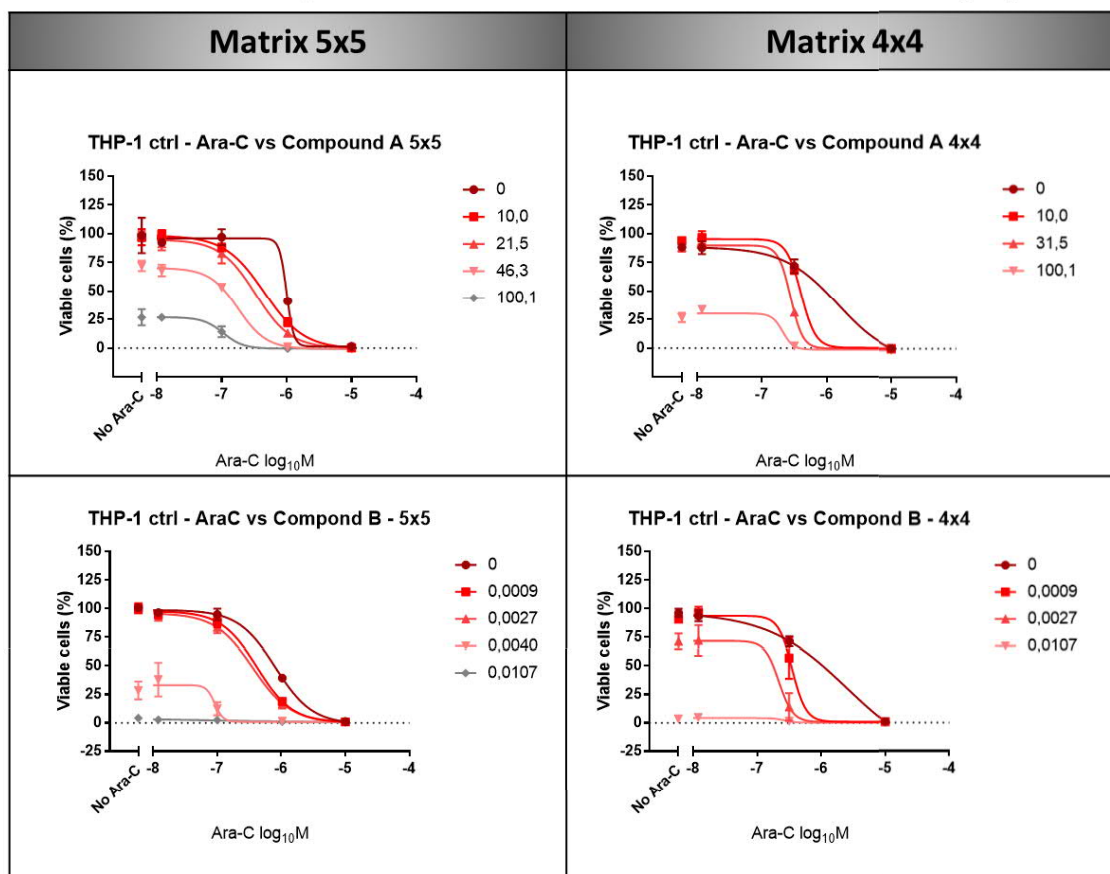
Figure 12, A dose-response curve with 5 data points is more accurate than with 4 data points. Proliferation inhibition analysis of drug combinations (above, ara-C and compound A; below, ara-C and compound B) in THP-1 ctrl cells. Left drug matrix 5x5, right drug matrix 4x4. Mean values ± SD of 4 independent experiments, each performed in duplicate.

2.4 Synergy between cytarabine and candidate SAMHD1 inhibitors is dependent upon SAMHD1 protein expression

To begin with, SAMHD1 protein expression levels in different hematological cell lines were determined. The aim was to use protein expression data, in subsequent correlation analysis, between drug synergy and SAMHD1 protein abundance. The second experiment with optimized drug matrix 5x5, aimed to evaluate the drug-drug interaction of ara-C and candidate SAMHD1 inhibitors (compounds A and B), in a panel of different hematological cancer cell lines (Table 1). Drug-drug interactions and possible synergy were examined with the three different synergy models, previously described (ZIP, HSA, and Bliss) and finally, the relationship between drug synergy and SAMHD1 protein abundance was evaluated using correlation analysis.

2.4.1 SAMHD1 expression in a panel of hematological cell lines

The aim of this part of my project was, to potentially establish a correlation between SAMHD1 expression and drug synergy. For this purpose, I determined the SAMHD1 protein expression levels amongst the chosen cell lines. Samples from different hematological cell lines were taken and protein levels were analyzed in a Western blot experiment (Figure 13). Cancer cell lines were categorized according to their SAMHD1 expression level: high expression (THP-1, KBM-7, MV-4-11), intermediate (HuT-78, K562, NB-4, MOLT-16) and low (HL-60, CCRF-CEM, Jurkat, MOLT-4).
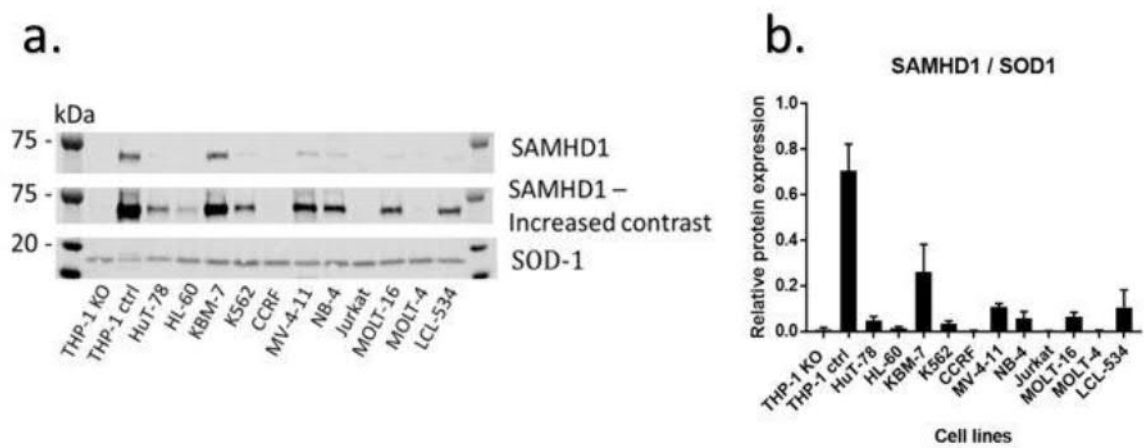
Figure 13, SAMHD1 expression differs between hematological cell lines. a, Western blot analysis of SAMHD1 expression in different hematological cell lines (for cell line details see Table 1). Representative of 4 blots shown. **b**, Quantification of SAMHD1 protein abundance. Values normalized to loading control SOD-1. Error bars indicate SD of 4 independent experiments.

2.4.2 Synergy between ara-C and inhibitors with optimized drug matrix

In a study with the optimized drug matrix across the cell line panel, first ara-C dose-responses from viability experiments were evaluated and then relative viability values were used to assess synergy with FIMM's online tool synergyfinder. As a result, synergistic interactions were detected with both drug combinations and greater synergistic interaction was observed in cell lines expressing more SAMHD1, compared to cell lines with lower expression (Figures 17 and 18). Therefore, a correlation analysis was performed to determine the relationship between drug synergy and SAMHD1 protein abundance. Examples from optimized drug matrix experiment (Figures 17 and 18) are presented after correlation results.

2.4.3 Correlation between SAMHD1 protein abundance and drug synergy in a panel of hematological cancer cell lines

Spearman correlation tests were used to determine a possible correlation between SAMHD1 protein abundance and drug synergy in a panel of cancer cell lines (n = 11). The results obtained indicated a weak positive correlation between SAMHD1 protein expression and drug synergy scores but a stronger correlation between expression and the most synergistic area value. Correlation plots are presented with 3 synergy models: ZIP, HSA, and Bliss respectively in Figures 14, 15 and 16, where correlation coefficient r is presented together with the P-value (two-tailed unpaired t-test, where P = <0.05 indicates statistical significance) to describe the linear relationship between SAMHD1 expression and synergy.

2.4.3.1 Correlation based on synergy results from ZIP model

In results obtained with the ZIP synergy model, both tested drug combinations showed positive correlation when synergy was evaluated with the most synergistic area method. With compound B the most synergistic area and SAMHD1 expression correlation coefficient was 0.7909 with P = 0.0055 and with compound A, r = 0.7818 with P = 0.0064 (Figure 14).

Correlation between SAMHD1 protein abundance (x) and drug synergy (y)

Figure 14, Spearman correlation of relative SAMHD1 protein abundance (SAMHD1/SOD1) and synergy values for combinations of ara-C and putative SAMHD1 inhibitors (A and B) based on ZIP synergy model. Correlation coefficient r is presented together with the p-value (two-tailed unpaired t-test, where p = <0.05 indicates statistical significance) to describe the linear relationship between SAMHD1 expression and synergy in a panel of cancer cell lines (n = 11). In ZIP results most synergistic area reflects stronger positive correlation than delta-score: with combination of ara-C + B, r = 0.7909 and P = 0.0055; with combination ara-C + A, r = 0.7818 and P = 0.0064. Data points with horizontal line and error bars are representing mean and SD from individual experiments; Western blot analysis (n = 4) and synergy studies, each performed in triplicate: THP-1 ctrl, n = 4; THP-1 KO, n = 4; HuT-78, n = 3; HL-60, n = 1; KBM-7, compound A n = 7, compound B n = 8; K562, compound A n = 4, compound B n = 5; CCRF-CEM, compound A n = 4, compound B n = 5; MV-4-11, compound A n = 4, compound B n = 5; NB-4, compound A n = 1, compound B n = 2; Jurkat, compound A n = 3, compound B n = 4; MOLT-4, compound A n = 3, compound B n = 4.

2.4.3.2 Correlation based on synergy results from HSA model

When synergy was evaluated with the HSA model, examined drug combinations showed a positive correlation with average synergy scores and even stronger correlation with the most synergistic area method. With compound B, the most synergistic area and SAMHD1 expression correlation coefficient was 0.7636 with P = 0.0086 and for compound A, r = 0.8455 with P = 0.0018 (Figure 15).
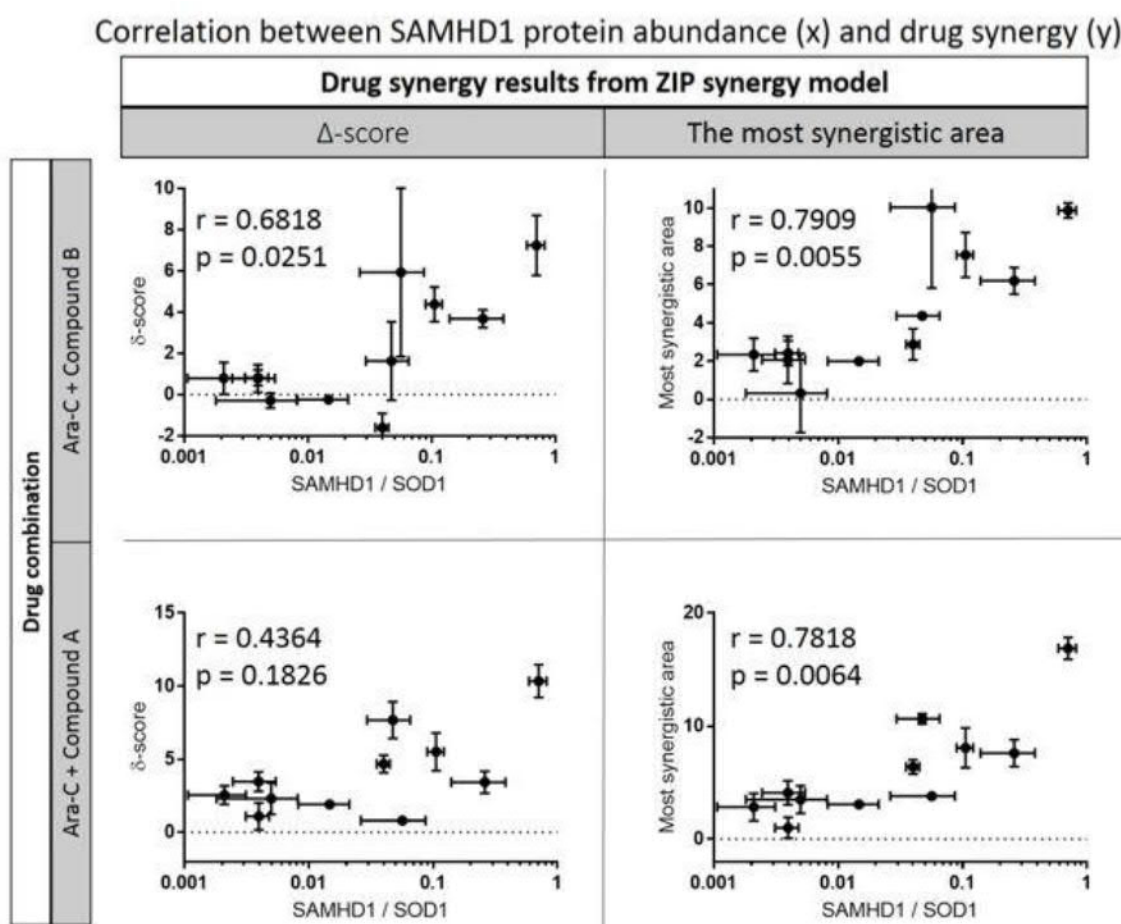
Figure 15, Spearman correlation of relative SAMHD1 protein abundance (SAMHD1/SOD1) and synergy values for combinations of ara-C and putative SAMHD1 inhibitors (A and B) based on the HSA synergy model. Correlation coefficient r is presented together with the p-value (two-tailed unpaired t-test, where p = <0.05 indicates statistical significance) to describe the linear relationship between SAMHD1 expression and synergy in a panel of cancer cell lines (n = 11). In HSA results most synergistic area reflects stronger positive correlation than delta-score: with combination of ara-C + B, r = 0.7636 and P = 0.0086; with combination ara-C + A, r = 0.8455 and P = 0.0018. However, also a weaker positive correlation is detected with an average synergy score (delta score) between both combinations; r = 0.6909 and P = 0.0226. Data points with horizontal line and error bars are representing mean and SD from individual experiments; Western blot analysis (n = 4) and synergy studies, each performed in triplicate: THP-1 ctrl, n = 4; THP-1 KO, n = 4; HuT-78, n = 3; HL-60, n = 1; KBM-7, compound A n = 7, compound B n = 8; K562, compound A n = 4, compound B n = 5; CCRF-CEM, compound A n = 4, compound B n = 5; MV-4-11, compound A n = 4, compound B n = 5; NB-4, compound A n = 1, compound B n = 2; Jurkat, compound A n = 3, compound B n = 4; MOLT-4, compound A n = 3, compound B n = 4.

2.4.3.3 Correlation based on synergy results from Bliss model

A correlation was also examined for Bliss synergy results and SAMHD1 expression. Again, the most synergistic area showed a stronger correlation: the most synergistic area score and SAMHD1 expression correlation coefficient for a combination of candidate inhibitor B and ara-C was 0.8364 with P = 0.0022 and for another combination (compound A + ara-C) r = 0.7727 with P = 0.0074 (Figure 16).
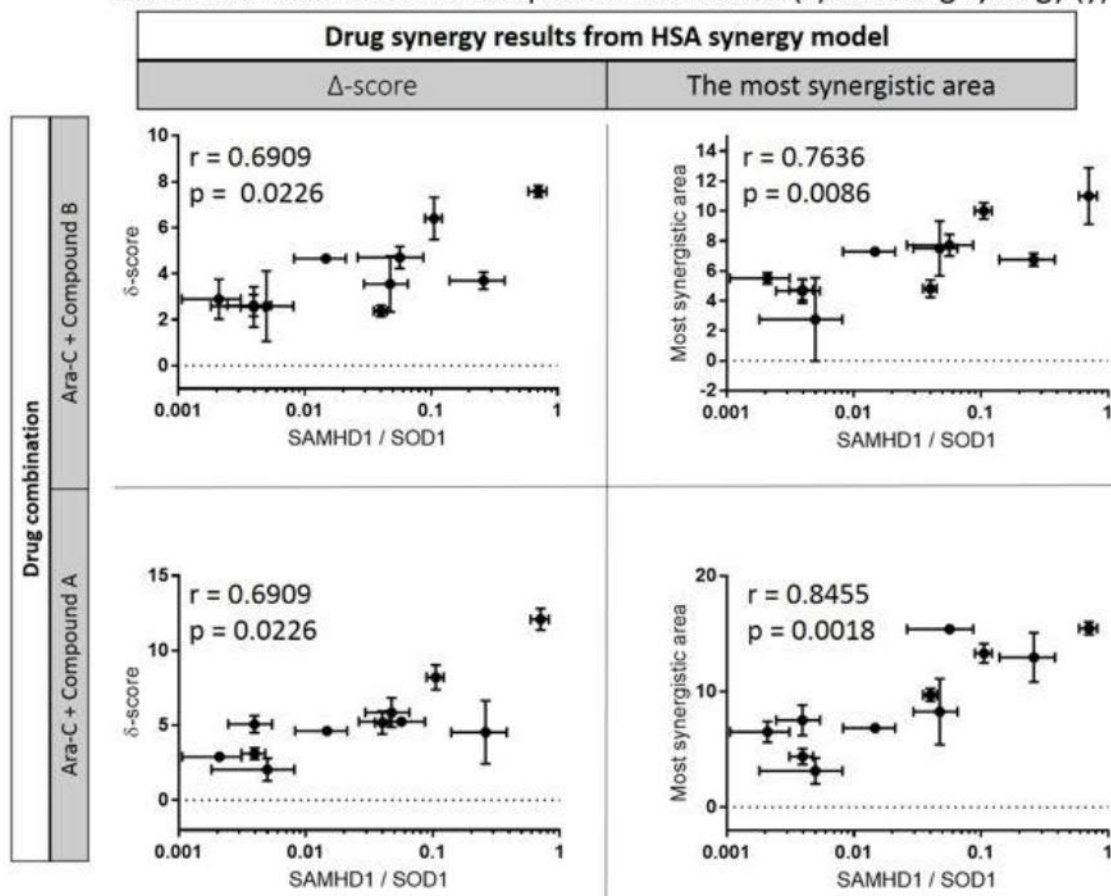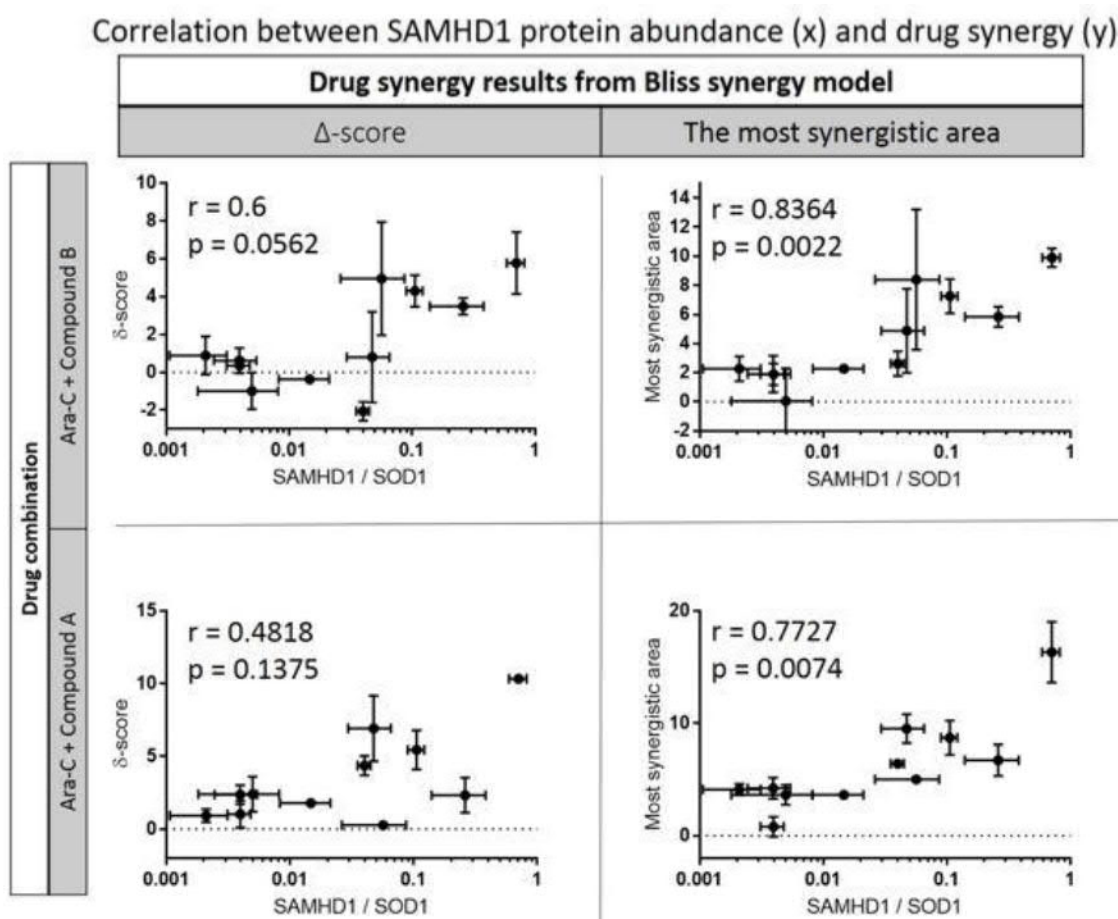

Figure 16, Spearman correlation of relative SAMHD1 protein abundance (SAMHD1/SOD1) and synergy values for combinations of ara-C and putative SAMHD1 inhibitors (A and B) based on Bliss synergy model. Correlation coefficient r is presented together with the p-value (two-tailed unpaired t-test, where p = <0.05 indicates statistical significance) to describe the linear relationship between SAMHD1 expression and synergy in a panel of cancer cell lines (n = 11). According to Bliss results, the most synergistic area reflects stronger positive correlation than delta-score: with combination of ara-C + B, r = 0.8364 and P = 0.0022; with combination ara-C + A, r = 0.7727 and P = 0.0074. Data points with horizontal line and error bars are representing mean and SD from individual experiments; Western blot analysis (n = 4) and synergy studies, each performed in triplicate: THP-1 ctrl, n = 4; THP-1 KO, n = 4; HuT-78, n = 3; HL-60, n = 1; KBM-7, compound A n = 7, compound B n = 8; K562, compound A

n = 4, compound B n = 5; CCRF-CEM, compound A n = 4, compound B n = 5; MV-4-11, compound A n = 4, compound B n = 5; NB-4, compound A n = 1, compound B n = 2; Jurkat, compound A n = 3, compound B n = 4; MOLT-4, compound A n = 3, compound B n = 4.

2.4.3.4 A summary of correlation results

In summary, relative SAMHD1 protein abundance was compared with drug synergy scores evaluated with different synergy methods. Synergy was evaluated with both delta-score and the most synergistic area value. Both methods displayed a positive correlation but it was more obvious with the most synergistic area method. Correlation between the most synergistic area and SAMHD1 expression, was statistically significant for both drug combinations (Table 3). To clarify the key results, correlation coefficient and p-values from ZIP, HSA and Bliss models are presented in table 3 together with different synergy evaluation methods.

Table 3, In a study with hematological malignancies (n = 11), SAMHD1 protein expression correlates with a combination of ara-C and putative SAMHD1 inhibitors measured with different synergy models. Correlation results from SAMHD1 expression and synergy experiments, where synergy was evaluated using average synergy, delta, scores and the most synergistic area-values. Spearman correlation of relative SAMHD1 protein abundance and drug synergy was examined for synergy results from different synergy models (ZIP, HSA, and Bliss). Results are shown with the correlation coefficient (r) and p-value (two-tailed unpaired t-test, where p = <0.05 indicates statistical significance, bold values).

Method for evaluating drug-drug interaction	Drug combination	Synergy model	Correlation coefficient (r)	P-value
Δ-score (An average synergy-score)	Ara-C + compound A	ZIP	0.4364	0.1826
		HSA	0.6909	**0.0226**
		Bliss	0.4818	0.1375
	Ara-C + compound B	ZIP	0.6816	**0.0251**
		HSA	0.6909	**0.0226**
		Bliss	0.6000	0.0562
The most synergistic area	Ara-C + compound A	ZIP	0.7818	**0.0064**
		HSA	0.8455	**0.0018**
		Bliss	0.7727	**0.0074**
	Ara-C + compound B	ZIP	0.7909	**0.0055**
		HSA	0.7636	**0.0086**
		Bliss	0.8364	**0.0022**

2.4.4 Examples from optimized drug matrix experiments

As an example from synergy experiments with optimized drug matrix, results with 2 cell lines are presented: MV-4-11 with intermediate SAMHD1 expression and MOLT-4 with low SAMHD1 expression (Figure 13).

MV-4-11 cell line was previously categorized to intermediate SAMHD1 expression group (Figure 13), but its SAMHD1 expression is still relatively high (less than THP-1 cells, but more than HuT-78 cells (Figure 17a). In the experiment with drug matrix 5x5, a combination of ara-C and compound B, enhanced ara-C cytotoxicity (Figure 17b) and produced a synergistic interaction (Figure 17c) in MV-4-11 cells. With this drug combination, an average of 5% more cell proliferation inhibition was observed over the expected additive response,

and with optimal drug concentrations, ara-C cytotoxicity was enhanced, even by 30%, compared to individual drug effects (Figure 17c).
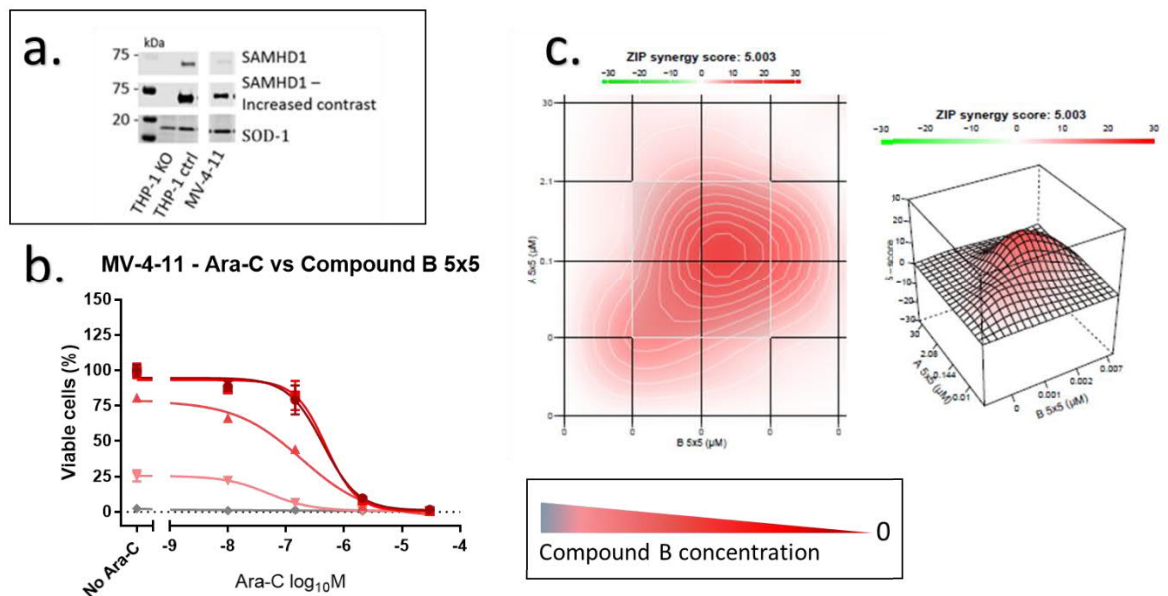

Figure 17, Compound B and ara-C synergistically inhibit cell proliferation in MV-4-11 cells. a, Western blot analysis of SAMHD1 expression in THP-1 ctrl, THP-1 SAMHD1 KO and MV-4-11 cell lines. A representative of 4 experiments shown. **b,** Viability by resazurin assay of MV-4-11 cells after 96 hours incubation with a drug combination of ara-C and compound B. Mean values ± SD of 3 independent experiments, performed in duplicate, are shown. **c,** Synergy landscape analysis of MV-4-11 cell line after 96 hours incubation with drug combination ara-C and compound B. Synergy results were obtained from FIMM's online tool synergyfinder using relative cell viability values. An average synergy score (delta-score) of 0 was considered as additive result, less than -5 as antagonistic and over 5 as synergistic (delta score for MV-4-11 cells was 5%). The most synergistic area is annotated with a pale square. Results are produced with ZIP synergy model.

In contrast to MV-4-11 cells, the MOLT-4 cell line has relatively low SAMHD1 expression (Figure 18a). Accordingly with our hypothesis, increasing the dose of inhibitor B did not potentiate ara-C cytotoxicity to the extent observed in MV-4-11 cells (Figures 17b and 18b). In line with lower SAMHD1 expression, a weak synergistic interaction was detected: ara-C combined with compound B enhanced ara-C cytotoxicity by 1,4% compared to the additive effect, and optimal drug concentrations enhanced ara-C cytotoxicity by 10-15%, in comparison to a corresponding, single drug concentrations (Figure 18c).
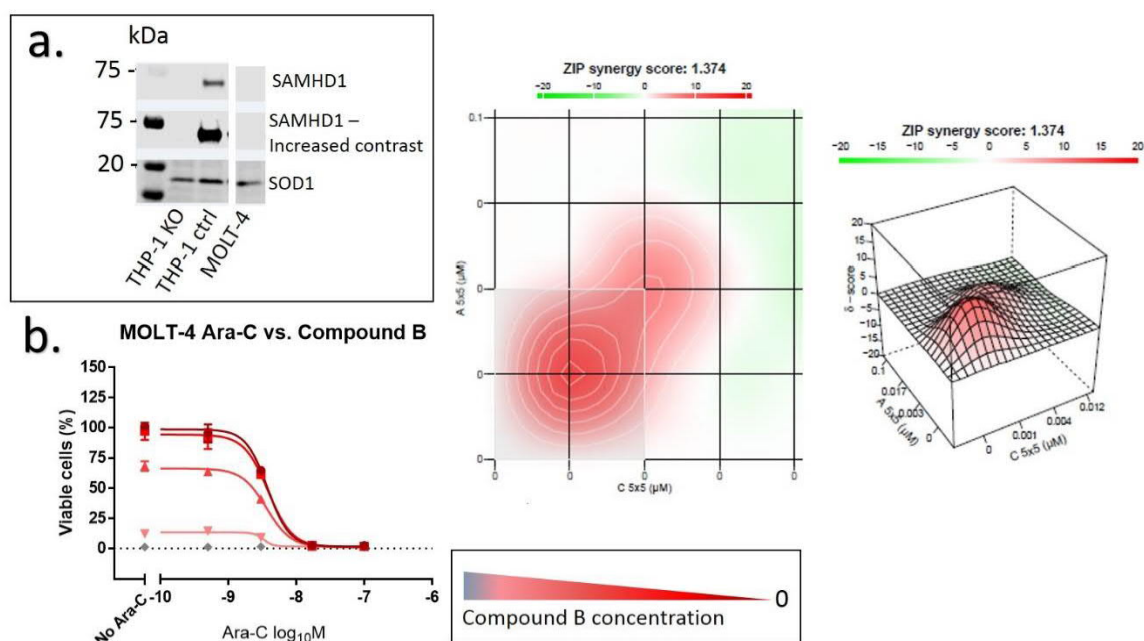
Figure 18, Compound B produces a weak synergistic effect in a combination with ara-C in MOLT-4 cell line. a, Western blot analysis of SAMHD1 expression in THP-1 ctrl, THP-1 SAMHD1 KO and MOLT-4 cell lines. A representative of 4 experiments shown. **b,** Viability by resazurin assay of MOLT-4 cells after 96 hours incubation with drug combination ara-C and compound B. Mean values ± SD of 3 independent experiments, performed in duplicate, are shown. **c,** Synergy landscape analysis of MOLT-4 cell line after 96 hours incubation with drug combination ara-C and compound B. Synergy results were obtained from FIMM's online tool synergyfinder using relative cell viability values. An average synergy score (delta-score) of 0 was considered as additive result, less than -5 as antagonistic and over 5 as synergistic (delta score for MOLT-4 cells was 1,4%). The most synergistic area is annotated with a pale square. Results are produced with ZIP synergy model

3. Discussion

SAMHD1 has been previously reported to limit the cytotoxicity of ara-C both *in vivo* and *in vitro* (Herold et al., 2017; Schneider et al., 2017). Based on these studies, I examined how SAMHD1 protein expression affects ara-C cytotoxicity in THP-1, HuT-78 and HL-60 cell lines. Indeed, results were consistent with previous studies: SAMHD1 deficient cell line variants displayed enhanced ara-C cytotoxicity in comparison to SAMHD1 proficient, control cell lines. Now the question was: could we improve ara-C cytotoxicity by creating a SAMHD1 deficient phenotype with chemical, small molecule, SAMHD1 inhibitors?

This question was tackled with drug-drug interaction (synergy) experiments, using two different drug combinations; ara-C combined with either candidate SAMHD1 inhibitor compound A or B. The aim was to examine the possibility to enhance ara-C cytotoxicity and we hypothesized that SAMHD1 limits the cytotoxic effect, hence both SAMHD1 proficient and deficient cell lines were used. From previous experiment, I chose THP-1 cell lines for the synergy experiments, as it provides the largest sensitivity scale amongst examined cell lines (THP-1, HuT-78 and HL-60). Drug-drug interactions were examined with three different synergy models (ZIP, HSA, and Bliss) and results from all models indicated a positive, synergistic, interaction for tested drug combinations in THP-1 control cells. The total effect produced by a combination of ara-C and candidate SAMHD1 inhibitors was greater than the additive drug effect and was therefore considered as synergistic. Drug synergy was evaluated using two different methods and both of them, average synergy across the drug matrix (delta score), and the most synergistic area, indicated positive, synergistic, drug-drug interaction in THP-1 control cells.

Obtained results validated our hypotheses: SAMHD1 proficient cells were expected to give a stronger ara-C response, because of higher SAMHD1 expression, respectively similar result, enhanced ara-C cytotoxicity, was not expected in THP-1 KO cells. Positive outcome with THP-1 cells resulted in subsequent experiments, investigating these drug combinations with other hematological malignances. However, using a lot of resources in synergy experiments was not economically reasonable, therefore assay development and optimization was required.

The synergy assay was further developed and optimized, aiming to reduce the amount of required reagents and increase the throughput, while conserving the robustness of our data. Optimization began by investigating dose-responses for ara-C, compound A and B in different hematological cancer cell lines. According to the individual dose-response results, a suitable drug concentration ranges were defined for subsequent synergy experiments. Additionally, SAMHD1 protein expression for different cancer cell lines was determined by western blot experiments, finally aiming to establish a correlation between SAMHD1 expression and synergistic drug-drug interactions.

Drug synergy matrix was optimized using THP-1 ctrl and THP-1 SAMHD1 KO cells. THP-1 ctrl cells has the highest SAMHD1 expression amongst examined cell lines, whereas SAMHD1 KO cells do not express SAMHD1. As before, using these cells in synergy optimization we expected to observe larger sensitivity than with HuT-78 or HL-60 cells. THP-1 proficient and deficient cells described drug-drug interactions in both high and low SAMHD1 expression levels, therefore our assay provided a large sensitivity scale and was optimal for screening purposes.

Synergy optimization experiment aimed to reduce the size of a drug concentration matrix. Four different drug matrices were examined: 8x8, 6x6, 5x5, and 4x4. Experiments were performed four times and results were compared using average synergy scores (delta-scores). In addition to comparison between drug matrices, we studied potential differences between used synergy models (ZIP, HSA, and Bliss). Surprisingly, each tested drug matrix produced a similar total result. Different synergy models displayed only small differences, as a matter of fact, these models were in line with each other, indicating a similar total interaction. To conclude, all examined drug matrices were suitable for synergy evaluation, however, drug concentration matrix 5x5 was chosen to be used in subsequent experiments. There were two main reasons affecting to this outcome. First of all, small matrices would be better for high throughput screening and therefore matrices 8x8 and 6x6 were excluded. Second reason was quality of a dose-response data. As dose-response information is used to create response surface models, which in turn are used to visualize more complex dose-response landscapes to describe drug-drug interaction (Yadav et al., 2015), it was necessary to evaluate the quality of dose-response results. If the dose-response information is not accurate enough, it will affect synergy results: inaccurate dose-response data does not necessarily give veritable synergy scores. The more data points are used, the more reliable dose-response curve becomes and therefore matrix 5x5 was more appropriate choice than matrix 4x4.

Optimized drug matrix, 5x5, was used to screen drug-drug interactions in a panel of hematological cancer cell lines and results indicated that cytotoxicity of ara-C was dependent on SAMHD1 protein abundancy. Combination treatment of ara-C and candidate SAMHD1 inhibitor produced more cytotoxic effect in cell lines expressing more SAMHD1 protein. Similar trend was observed with each different synergy model (ZIP, HSA, and Bliss) and when synergy was evaluated

either with an average synergy (delta-score) or the most synergistic area method, results were consistent. These findings suggested a potential positive correlation between SAMHD1 protein abundance and drug synergy and finally, this relationship was examined using Spearman correlation analysis.

As drug-drug interactions were demonstrated with average synergy and the most synergistic area method, synergy scores from both methods were blotted against SAMHD1 protein expression data. After analyzing the correlation, we compared the linear correlation between used synergy methods, average synergy against the most synergistic area, to determine possible differences between used methods. In this comparison, the most synergistic area plotted with SAMHD1 expression indicated a stronger positive correlation than average synergy. In fact, results from all used synergy models showed a positive correlation when the most synergistic area –scores for both drug combinations (ara-C+A / ara-C+B) were compared against SAMHD1 protein abundance. Additionally, positive correlation was detected with average synergy scores (delta scores), however, synergy evaluated by this method did not show significant results with all synergy models. There was a positive correlation between SAMHD1 protein abundance and average synergy scores, for a combination of ara-C and compound A when drug-drug interaction was evaluated with HSA model. Another drug combination (ara-C and compound B) indicated a significant positive correlation when synergy was evaluated with ZIP or HSA models.

As a conclusion, both drug combinations (ara-C and candidate SAMHD1 inhibitors A or B) showed positive, synergistic, drug-drug interaction in experiment using drug matrix 5x5. Spearman correlation analysis suggested significant correlation more often than insignificant, altogether correlation analysis was performed for 12 cases and 9 of them showed significant result (Table 3). Additionally, the most synergistic area showed more significant correlations (6/6) than average synergy method (3/3) (Table 3).

3.1 Reviewing the methods

Combination treatment in this work was evaluated with 3 different synergy models (ZIP, HSA and Bliss), using an average synergy score and the most synergistic area methods. However, additional methods for evaluating drug-drug interactions are available and the most widely used method is the Chou-Talalay method, which uses combination index –value (CI) to describe synergistic interactions

between two or more drugs (T.C Chou, 1984; Roell et al., 2017). Although CI is commonly used, it has limitations and for example nonlinear interaction often leads to incorrect conclusions and evaluating interactions without toxic effect is challenging (Roell et al., 2017). These factors are limiting the usefulness of CI method and create errors in the interpretations of the results. An average synergy and the most synergistic area methods, however, can be used to detect interactions with toxic effect (Foucquier and Guedj, 2015; Yadav et al., 2015). Because different synergy models are based on different assumptions, it is necessary to compare results from different models to increase reliability of the results. For this purpose, I used three different models and two different methods to evaluate drug-drug interactions.

Another challenge was assay development. Optimization led to drug matrix 5x5 but what are the benefits and risks using this drug matrix in synergy experiments? It is important to consider the reliability of the results obtained using the optimized matrix, where 5 data points were used. Is the size of a drug matrix a limiting factor? According to my results, drug-drug interaction was similar between tested drug matrices and therefore suggests that small matrices, like 5x5, are usable to determine drug-drug interactions. However, one can confirm already obtained data with more precise drug concentration matrix e.g. 8x8, if needed. With 8 concentrations reliability is increased when dose-response curve is based on several data points, and this reduces errors and makes average synergy score more accurate. The most synergistic area is based on the highest observed effect (Yadav et al., 2015), therefore it is not as sensitive to dose-response errors as delta score.

Differences or errors in results might occur due to the assay used, in this case for example size of a drug matrix, as discussed, or then differences in used cell lines. As I used a cell-based assay, differences between cell lines should be considered more carefully. Now optimization was based on THP-1 cell line, though SAMHD1 proficient and deficient lines were used, there might still be variation between different cell lines. Although the used assay was sensitive and capable to detect different drug-drug interactions, the problem might be in cell lines or how they were prepared for the experiment. Can we trust the results or compare the observed effects if we do not know cell lines normal behavior? One important aspect is cell proliferation rate, how rapidly cells are dividing. When I tested drug

combinations in a panel of hematological malignancies I did not take into account the natural variation between cell lines e.g. variation in proliferation rates.

If combination treatment is evaluated, then it would be important to ensure the same drug exposure time, standardize the whole experiment. Cytotoxic drug effect might be related to a specific cell cycle phase and if a cell line is exposed to drug more often than another cell line, then the cytotoxic outcome is more probable. For example, the active form of ara-C, ara-CTP, damages DNA in the S-phase when DNA is synthesized. Comparing CCRF-CEM and NB-4 cell lines, if rapidly proliferating CCRF-CEM cells and more slowly proliferating NB-4 cells are incubated with ara-C for the same period of time, the total exposure to cytotoxic agent would not be equal. However, the total exposure becomes different also due to the presence of SAMHD1. As stated in the introduction, this enzyme converts active compound back to its inactive form (Herold et al., 2017), then a cell line with low protein levels is exposed to cytotoxic ara-CTP more frequently, compared to a cell line with more abundant SAMHD1. If putative SAMHD1 inhibitor is used, it equilibrates the exposure time to cytotoxic drug between different cell lines. Also, a specific drug concentration with longer incubation time stabilizes the potential differences in total drug exposure.

As a final result, a positive correlation between SAMHD1 protein abundance and drug synergy was detected. As discussed earlier, this correlation was based on SAMHD1 expression and drug synergy results, but the level of protein expression does not necessarily guarantee or correlate with enzymatic activity. Though, Herold et al., have showed that higher SAMHD1 mRNA levels are associated with poor ara-C response in patients, in comparison to lower mRNA status (Herold et al., 2017). Protein expression quantified with western blot does not necessarily indicate significant enzyme, in this case, SAMHD1 activity. Rentoft et al. studied colon cancer and disclosed that some *SAMHD1* mutations reduced the hydrolytic activity against dNTPs (Rentoft et al., 2016). Gene activity differs amongst individuals and for example gene mutations or SAMHD1 splice variants, might have an effect to SAMHD1 activity, and hence to ara-C response (Shi et al., 2014; Rice et al., 2009).

Continuing with *SAMHD1* status: if the gene is active it does not necessarily guarantee an active gene product, e.g. enzyme, and that is why studying transcriptomic activity, comparing gene expression with protein expression, is

important. Transcriptional activity of a specific gene *loci* could be examined with measuring mRNA levels and in contrast to this thesis work, reliability of the *in vitro* results could have been improved by comparing both mRNA and protein levels with synergy results. On the other hand, if we already have *in vivo* evidence, is it necessary to use additional resources to confirm it with new *in vitro* studies? After all, SAMHD1 has already been identified as a risk factor and a potential prognostic marker to determine ara-C responses in AML patients (Herold et al., 2017a; Schneider et al., 2017).

3.2 Potentiating the efficacy of current AML therapy – AML treatment now and tomorrow

AML treatment affects patient well-being: on one hand, we can improve the patient's quality of life, on the other hand, treatment can be harmful to a patient. Current treatment options are lacking efficacy, there are patients who are not responding, and additionally, cancer treatment can be an exhausting experience and may involve severe adverse events (e.g. drug side-effects). There are unmet need for more effective and tolerable treatment options, especially amongst elderly patients (Bell et al., 2018).

Cancer treatment and research have improved a lot during the last decades and new therapeutic options have been discovered and developed continuously. However, cancer is difficult to treat due to its heterogeneity and constant evolution. Nowadays cancer treatment is developing towards more precise, individualized, treatment and there are clinics where the treatment is completely tailored to a patient, taking into account, for example, genetic profile of the individual. This genomic-guided therapy has been linked to improved patient outcomes (Senft et al., 2017) and is important due to inter- and intrapatient genomic heterogeneity.

In this thesis, I examined an option where current AML treatment could be potentiated. This is one approach, where the aim is to enhance the properties of the original compound, making it more efficient, simultaneously reducing its possible side-effects. When candidate inhibitors were used, less ara-C was needed to achieve the therapeutic effect in comparison to incubation with ara-C alone. Using potentiating drug combination, we can reduce the amount of needed individual drugs and therefore, we hope to minimize their potential drug-related adverse-events. Especially in cancer treatment: chemotherapeutic agents are

usually quite "heavy drugs" and the less we can use them, the better it will be for the wellbeing of the patient. In this case, we would add another drug and combine it with ara-C. Now side-effects of ara-C (such as: headache, dizziness, severe nausea and vomiting) are probably less common, because of the reduced amount of the drug, but what about the new drug and its side-effects. What can inhibition of SAMHD1 cause?

SAMHD1 gives protection against viral infections. When dNTP levels are low, viral replication becomes more difficult (Baldauf et al., 2013), so if we inhibit SAMHD1 does this make us more vulnerable to viral infections? This question becomes important because AML is hematological cancer where malignant cells arise from the bone marrow. This leads to a lack of functional neutrophils and as a total effect, AML patient's immune system becomes depressed by the disease itself. Then what is the combined effect of already weak immune system together with other immunosuppressive medications, is this really a significant concern? Probably not, of course with some individuals this might be an important question, but there are also other defense mechanisms against viral infections (Alberts et al., 2008). These mechanisms are including for example: immunization responses of innate immunity and another intracellular defense mechanisms like: intracellular pathogen receptors, genome destruction, removing genomic material and destruction of nucleic acids, for example through interferon (IFN) mediated pathway.

Another considerable aspect is polypharmacy. Is it really a good idea to increase the number of drugs as we should try to reduce the drug overload? For instance, elderly people are often exposed to many drugs, and when more drugs are used simultaneously, that increases the probability of unwanted drug-drug interactions and might be that drugs are preventing each other's functions or then they might potentiate each other's, either way, the outcome might not be what originally expected.

Maybe the most important question deals with cytotoxic drug ara-C. Drug-drug interaction between ara-C and putative inhibitors is pharmacokinetically potentiative, where therapeutic activity, of ara-C is enhanced by inhibiting detoxification of the biologically active agent, ara-CTP. If we use combination treatment to enhance the effect of already cytotoxic drug, is this reasonable approach? Again this is a question of risk assessment, what are the possible

benefits in comparison to potential risks. Because potentiation of ara-C cytotoxicity is more efficient in cells where SAMHD1 protein is more abundant, using a combination treatment is reasonable with patients that have higher SAMHD1 protein abundance.

In the near future, new treatment options for AML should be available to patients and new immunological and stem cell targeted therapies are currently under research. Leukemic stem cells (LSCs) might explain why disease relapses, and for instance, LSCs genetic background has been examined with *in silico* assay to determine gene-expression signatures, then information can be combined with *in vitro* screening, intending to identify new compounds (Laverdière et al., 2018). In addition to drug-gene interactions, immunological treatments are also under examination. Current role of tumor microenvironment in AML is not yet completely understood but immunological treatments, like checkpoint modulator anti-PD-1 (programmed cell death protein 1) nivolumab, has been promising anti-cancer strategy also in AML studies (Lamble et al., 2018). However, immunological treatments might be quite expensive, whereas small molecules are usually cheaper and user-friendlier.

3.3 Conclusions

According to current knowledge, there are patients with high SAMHD1 mRNA levels and this has been linked to weak ara-C treatment response (Herold et al., 2017). In this work: I optimized a cell-based assay for evaluating drug-drug interactions in a panel of hematological cancer cell lines. Additionally, I used the assay to evaluate drug-drug interaction between SAMHD1 inhibitor candidates (compounds A and B) and ara-C and finally accessed a positive correlation between SAMHD1 protein abundance and drug synergy for combination of ara-C and both candidate SAMHD1 inhibitors, examined in a panel of hematological cancer cell lines.

Herold et al. demonstrated that lower ara-C sensitivity is positively correlating with SAMHD1 mRNA expression in a panel of hematological cell lines and additionally, they detected association between low SAMHD1 expression and enhanced ara-C treatment response in AML patients (Herold et al., 2017). Taken together, the positive correlation evidence from this thesis work supported the

rationale of individualized treatment option: combination of ara-C and SAMHD1 inhibitor should improve treatment outcome more efficiently in AML patients with high SAMHD1 activity.

This topic is clinically important as current AML treatment is not effective enough and especially in elderly people not tolerable enough, additionally, the lack of efficacy increases possibility for a relapse. We believe that the efficacy of current ara-C treatment could be potentiated by combining ara-C with a SAMHD1 deficient phenotype using small molecule inhibitors. This would then result in enhanced cytotoxicity of ara-C, potentially leading to improved therapeutical outcomes. As stated before, a need for combination treatment of ara-C and inhibitor becomes even more important in patients with high SAMHD1 protein expression. However, high expression does not guarantee significantly high enzyme activity. Therefore, this approach should be a part of individualized cancer treatment. Firstly, we should evaluate patient's SAMHD1 protein expression and activity, then a need for combination treatment could be considered as a part of treatment's risk-benefit evaluation. Consequently, biomarker-based patient identification should prevent unnecessary toxicity and improve overall cost-effectiveness of the treatment.

4. Materials and Methods
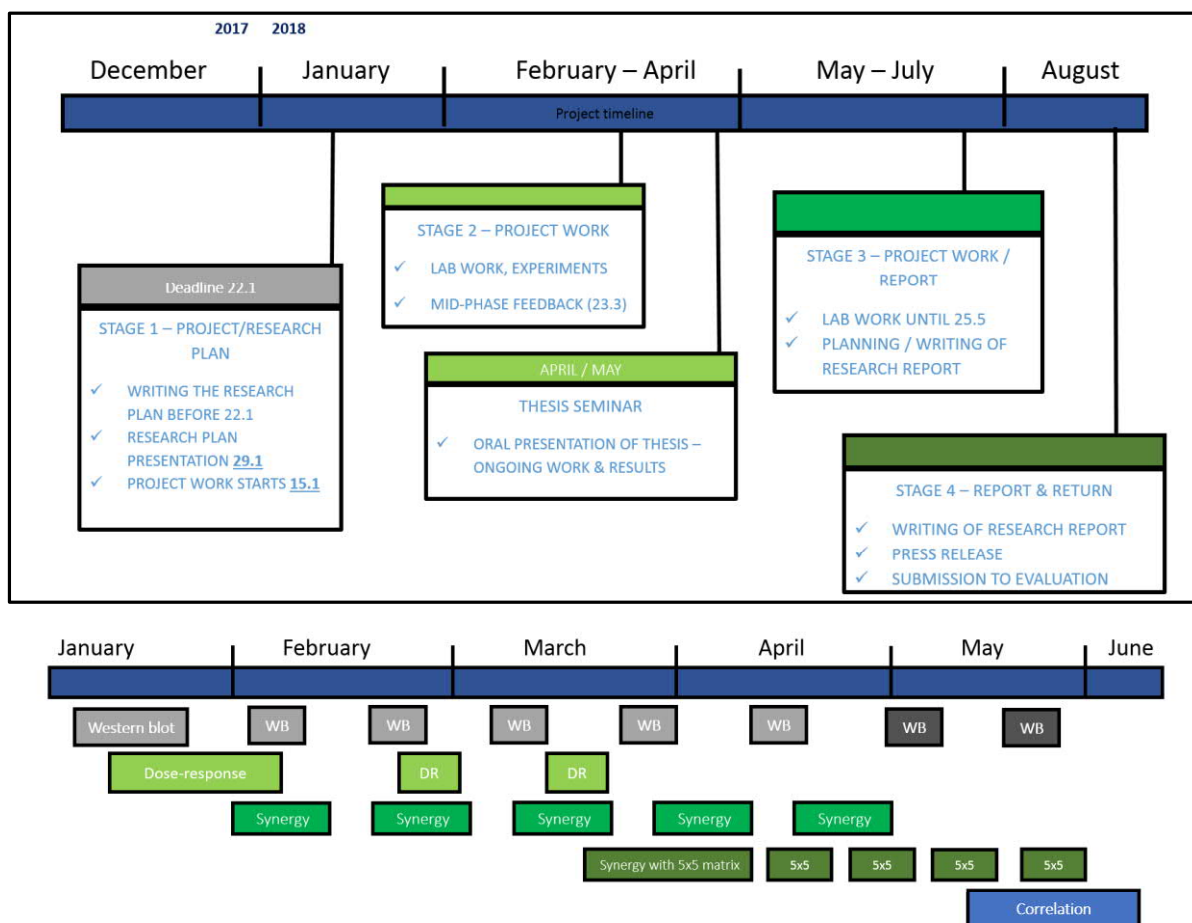
4.1 Project timeline

Figure 19, Project timeline. Above description of different stages of this MSc thesis project. Below more detailed timeline about the main experimental lab parts. At the end of the work, a couple of additional western blot experiments were performed where we examined DNA damage and cell death signals after combination treatment, results are not presented in this thesis (dark grey wb-boxes).

4.2 Cell lines and cell culture

Cell lines were purchased from ATCC and cultured in two different cell culture media were. THP-1, HuT-78, HL-60 cells and their CRISPR/Cas9´-generated (SAMHD1 KO) derivatives were cultured in Iscove's Modified Dulbecco's Medium (IMDM; GE Healthcare). All other cell lines (K562, CCRF-CEM, MV-4-11, KBM-7, MOLT-4, MOLT-16, NB-4, Jurkat and LCL-534) were cultured in Roswell Park Memorial Institute medium (RPMI 1640 GlutaMax; ThermoFisher Scientific). Media were supplemented with antibiotics 1% (100 U/ml penicillin – 100 µg/ml streptomycin; ThermoFisher Scientific) and 10% heat-inactivated fetal bovine

serum (FBS, ThermoFisher Scientific). In synergy experiments cells were resuspended with media containing 5% FBS, instead of 10%. Cell lines were regularly monitored and tested negative for the presence of mycoplasma using a commercial biochemical test (MycoAlert, Lonza). Culturing densities were between $2x10^5 - 1x10^6$ cells/ml, additionally a humidified incubator was used (37 °C with 5% CO_2).

4.3 Compounds

Cytarabine was purchased from Sigma Aldrich, Sweden (cat no. C1768). Inhibitor details are not available. Compounds were prepared as stock solutions in DMSO and they were either stored at -20 °C or prepared fresh. Compounds were diluted in DMSO.

4.4 Controls

Both positive and negative controls were used in viability experiments. Wells containing cells in culturing media were considered as positive control (100% viability) and wells with media only were considered as negative control (0% viability). Controls were used in normalization: fluorescence intensities from viability measurement were normalized to the average of control plates on the same plate. DMSO volume was normalized across the plate, not exceeding a total volume of 500 nl/well.

4.5 Dose-response experiments

Dose-response experiments were performed to determine the concentration ranges to be used in synergy experiments. Half-log diluted concentrations (total 8 concentrations), in 96-well plates (U-bottom 96-well plates, ThermoFisher Scientific), were used to obtain a complete concentration response curve with each individual compound. Each drug was performed in triplicate and for ara-C concentrations from 30 µM to 0.014 µM were used. 2 cell lines were placed on 1 plate and 5000 cells/well were incubated together with examined compounds 96h. Viability was measured with resazurin assay after 8h incubation with the reagent (resazurin sodium salt (Sigma Aldrich, cat no. R7017) 0.06 mg/ml diluted in PBS, measured with Hidex Sense plate reader (530-570/590-620 nm - ex/em)).

4.6 Drug synergy

384-well plates (Corning, REF 3764) were used in synergy experiments. Compounds were first dispensed with Tecan D300e Digital Dispenser using the

Synergy wizard in the D300e Control Software. DMSO volume was normalized across the plate, not exceeding a total volume of 500nl/well. Cells were seeded to plates with MultiDrop (ThermoFisher Scientific), final density was 1000 cells/well/total V = 50 µl and incubated 96 h before starting cell viability measurement with resazurin assay (10 µl resazurin sodium salt (Sigma Aldrich, cat no. R7017) 0.06 mg/ml diluted in PBS, measured with Hidex Sense plate reader (530-570/590-620 nm - ex/em) after 8h incubation). All experiments were performed in duplicate and the average, relative viability, was used for dose-response matrix analysis with online tool Synergyfinder (Yadav et al., 2015).

Dose-response landscapes from ZIP, HSA and Bliss models were generated and average synergy scores (delta-scores) together with the most synergistic area values were calculated. The average synergy score, delta, describes the total inhibition in percentage over the expected effect, for example delta score 3,2 indicates that with used concentrations drug combination produces an average of 3,2% more proliferation inhibition compared to additive, individual drug, responses. Score of zero describes that there is no interaction and negative delta-score means antagonistic interaction. Additionally a delta score >5 was categorized as strong synergy and accordingly a delta score <5 as strong antagonism (Yadav et al., 2015). Each synergy model is based upon a different assumption: HSA model compares the effects of combination treatment and the highest monotherapy, Bliss model assumes that two drugs are not interacting, instead they are acting independently on the phenotype, and ZIP model is combining Bliss and Loewe models and assumes that two drugs do not potentiate each other.

4.7 Protein measurement (Western blot)

For western blot analysis 4ml samples were taken from cell cultures, cells were counted with automatic cell counter (BioRad TC20) and 2x10^6 cells were harvested for western. Cells were washed with 1ml PBS and centrifuged 500g/5min during the washes. Washed cells were centrifuged 1000g/5min and pellet was stored on ice. Cell lysis buffer was prepared according to formula presented in table 3 and 50µl/sample was used.

Table 4, Lysis buffer for western blot. Protease inhibitor cocktail (Roche - 04693159061), phosphatase inhibitor (ThermoFisher Scientific - 1861277).

	1x	Stock	Dilution	Volumes for 1ml
Tris HCl, pH 8	50 mM	2 M (pH 7.5)	1/40	25µl
NaCl	150 mM	5 M	1/33.3	30µl
EDTA	1 mM	0.5 M	1/500	2µl
TX100	1%	10 %	1/10	100µl
SDS	0.1%	20 %	1/200	5µl
Protease i		10x	1/10	100µl
Phos i		100x	1/100	10µl
H$_2$O				728µl

Protein concentration of cell lysates was measured with Pierce BCA assay (Protein Assay Kit, ThermoFisher Scientific), with standard curve from 2000 µg/ml to 0 and experiment was run in duplicate. Luminescence was measured with Hidex Sense plate reader (570nm). According to BCA assay, equal amount of protein was calculated to be used. Samples and buffers were prepared: Laemmli Sample Buffer (BioRad), loading buffer (LB+1M DTT, final c=100nM) and running buffer (BioRad TGS buffer (1610732), 10xTRIS/Glysine SDS buffer: 100ml buffer + 900 ml H$_2$O). Prior to running, samples were denatured at 80 °C 3 min. 4-5% TGX gels were used (Bio-Rad Mini Protean TGX) and gels were ran with BioRad running chamber, 120V 1-2h. Transfer to nitrocellulose membrane with Transblot Turbo (BioRad) 1,3A, 25V, 7min. Blocking was done with odyssey blocking buffer (LICOR 927-50000/TBS tween (0.1%)). Primary Ab O/N, 2ml in 50ml falcon tube (SAMHD1 rabbit Ab 1:2000 (Abcam, ab177462) and loading controls SOD-1 (Mouse Ab 1:20000, Santa Cruz, sc-11407) and beta actin (Mouse Ab 1:4000 (Abcam, ab49900)). Washes after primary antibody with TBST (tris-buffered saline with 0.1% Tween-20). Secondary detection antibodies: R800 for SAMHD1 and M680 for SOD-1 and beta-actin) 1:10000, incubation 1h dark RT. Images were taken with LI-COR Odyssey image system (channels 700 and 800) and band intensities were quantified with ImageStudio Lite software (LI-COR Bioscience).

4.7 Statistical methods

Dose-response curves, One-way analysis of variance (ANOVA) and Spearman correlations (nonparametric) were performed with Prism 7 (GraphPad Software). Statistical significance was demonstrated with P-value and $P = <0.05$ indicated statistical significance.

5. Acknowledgements

6. Abbreviations

AGS	Aicardi–Goutières syndrome
AML	Acute Myeloid Leukemia
Ara-C	Arabinosylcytosine or Cytarabine
CI	combination index
dC	Deoxycytidine
dGTP	Deoxyguanosine triphosphate
dN	Deoxynucleoside
dNTP	Deoxyribonucleoside triphosphate
FIMM	Institute for Molecular Medicine Finland
HIV-1	Human Immunodeficiency Virus, type 1
HSA	Highest Single Agent
HTS	High throughput screening
IC$_{50}$	Half maximal inhibitory concentration.
KO	Knockout

LSC	leukemic stem cell
mRNA	Messenger RNA
PPPi	Inorganic triphosphate
RNA	Ribonucleic acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAMHD1	Sterile alpha motif (SAM) and histidine/aspartic acid (HD) domain-containing protein 1
SCT	Stem Cell Transplant
SD	Standard deviation
Vpx	Viral protein x
ZIP	Zero Interaction Potency

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8. Appendices
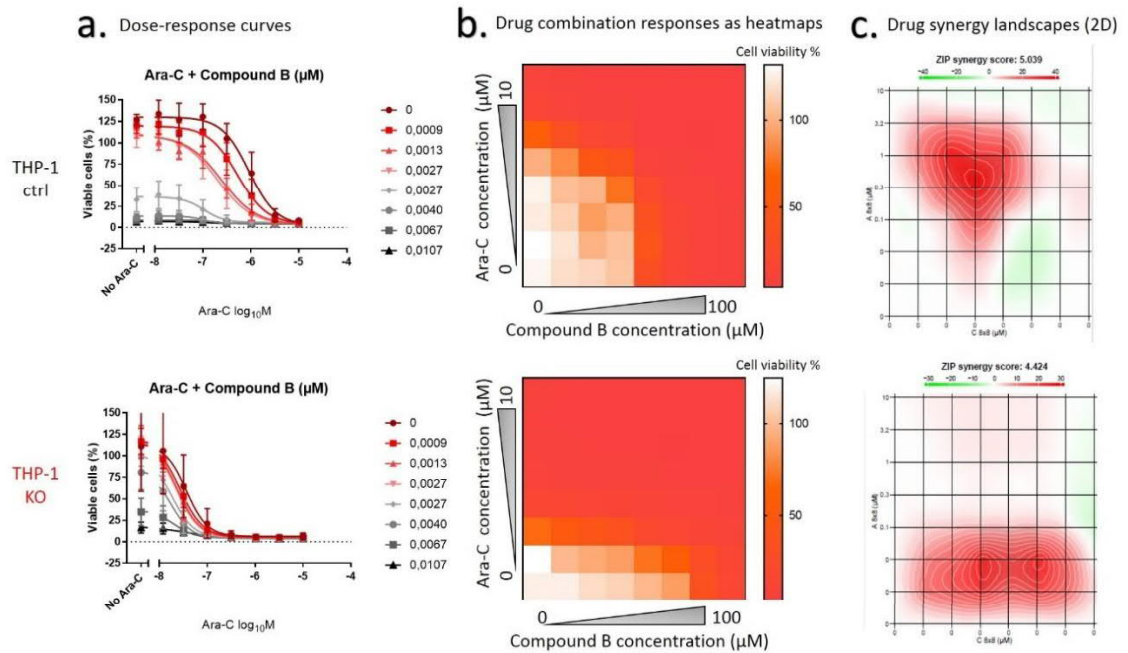
Appendix 1, A drug combination of potential SAMHD1 inhibitor (compound B) and ara-C is synergistic and enhances the ara-C cytotoxicity in THP-1 ctrl cells. **a**, Proliferation inhibition analysis of combination treatment ara-C + compound A in THP-1 SAMHD1 proficient (ctrl) and deficient (KO) cells. Viability was measured with resazurin assay after 96 hours drug incubation. Relative viability from dose-responses was analyzed with FIMM's online tool synergyfinder, and results are presented with inhibition heatmaps and synergy landscapes. **b**, Drug combination responses as heatmaps for combination ara-C and compound B in THP-1 SAMHD1 proficient (ctrl) and deficient (KO) cells. Grey triangles indicate concentration (μM) of ara-C (y-axis) and compound B (x-axis) in a coordinate system where the origin is equal to zero.**c**, Synergy landscapes (2D) for THP-1 SAMHD1 proficient (ctrl) and deficient (KO) cells after 96 hours incubation with drug combination of ara-C (y-axis) and compound B (x-axis). An average synergy score (delta-score) of 0 was considered as additive result, less than -5 as antagonistic and over 5 as synergistic (delta score for KO cells was 4,4% and for ctrl cells 5%).
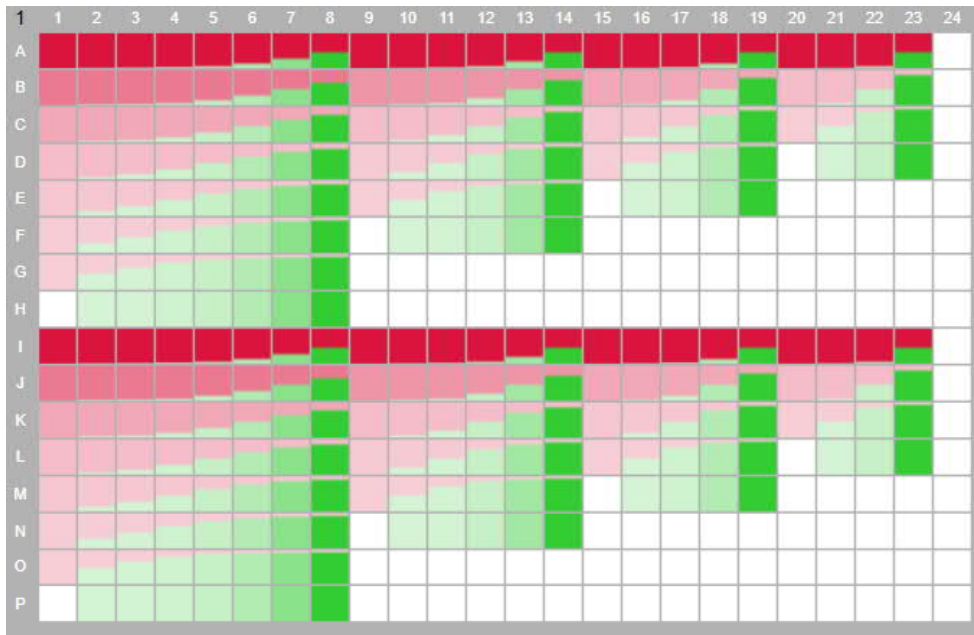
Appendix 2, Plate layout of drug matrix optimization experiments. 4 different drug matrices were used in duplicates: 8x8 (1-8), 6x6 (9-14), 5x5 (15-19) and 4x4 (20-23), column 24 was used as negative control (0% viability) containing cell culture media.
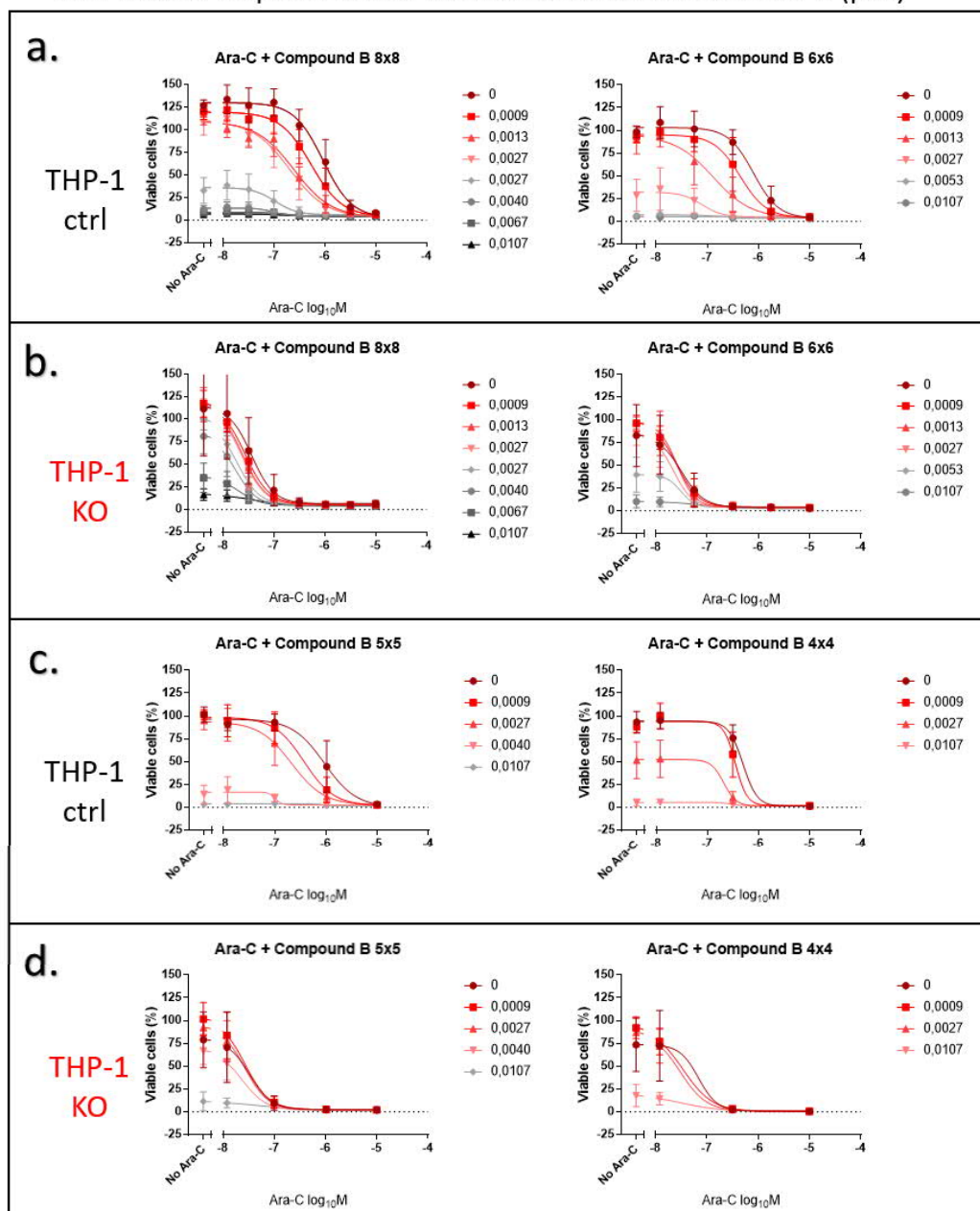
Appendix 3, Combined ara-C dose-response curves from synergy optimization study with candidate SAMHD1 inhibitor B (µM). Ara-C dose-response curves for THP-1 ctrl and SAMHD1 KO cells. Cell viability was determined by resazurin assay of THP-1 ctrl (**a,c**) and SAMHD1 KO (**b,d**) cells, following 96 hours incubation with a combination of ara-C and compound B. Drug matrices (**a,b**) 8x8, 6x6; (**c,d**) 5x5 and 4x4, were compared. Compound B concentrations are indicated in µM. Mean values ± SD of 4 independent experiments, performed in duplicate (plate layout in Appendix 2).
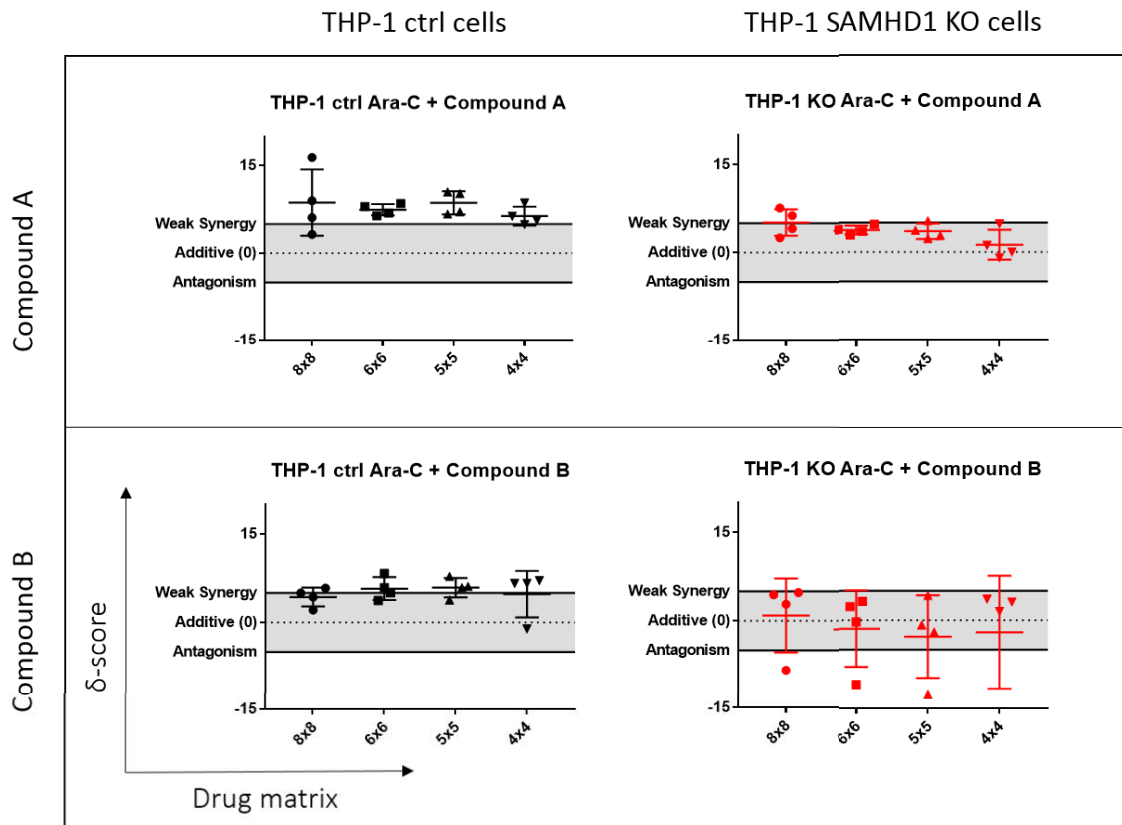
Appendix 4, Drug matrix optimization results from the Bliss model. Relative dose-response values from resazurin viability experiments (n = 4) of THP-1 ctrl and SAMHD1 KO cells after 96 hours incubation with drug combinations (ara-C and either compound A or B), were used to obtain an average synergy scores (delta scores – y-axis) from FIMM's online tool synergyfinder. On the top part of the figure: average synergy scores for drug combination ara-C + compound A and at the lower part of the figure: combination ara-C + compound B. Individual data points indicate mean values from 4 independent experiments, each performed in duplicate, error bars indicate SD. THP-1 ctrl cells are represented in black and THP-1 SAMHD1 KO cells in red. Drug matrices were compared with an average synergy scores (delta-scores) and delta equal to zero was considered as an additive response, above 5 as a synergistic and less than -5 as an antagonistic interaction, the grey rectangle is indicating the area between antagonistic and weak synergistic interaction (delta from -5 to 5). Statistical significances between drug matrices are presented in Table 2.